Comparing chemical analysis with literature studies to identify micropollutants to be treated or upstream source controlled in a catchment of Copenhagen (DK)

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and biochemical responses. For example, a number of highly replicated fish life-cycle and multi-generational tests have examined growth, development and reproductive success in combination with biomarker endpoints such as vitellogenin, GSI, and gonad histology. Analyses of sperm quality, gonad development, VTG, genomic markers, and other non-traditional endpoints have also been reported in the literature, often in isolation of a link with more traditional endpoints. In determining the utility and relevance of these studies and their novel endpoints in a risk assessment framework, a clear understanding of the relationship between biomarker and sublethal findings, which may or may not be adverse, to population relevant assessment endpoints must be established and data quality and reliability must be determined. The evaluation of data, including both traditional and non-traditional endpoints should be done in a weight of evidence approach in order to reach a scientifically defensible risk determination. In so doing, potential adverse impacts of the compound can be defined and used as measures of effects that are directly related to assessment endpoints for a sound and robust risk assessment. A case study describing this approach for the evaluation and use of both traditional and non-traditional endpoints will be presented using examples from the extensive aquatic database for BPA.

RA23P - Wastewater effluent discharges: chemical characterisation and understanding potential risks in receiving waters

TH 303

Risk assessment of WWTPs water effluents using fuzzy logic

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This paper presents a new methodology to assess the risk of water effluents based on fuzzy logic, a very well-known theory to treat uncertainty and vagueness, especially in the environmental field where data many times are not fully available. The method has been tested using the effluent's pollution data coming from 22 waste-water treatment plants (WWTPs) located in Catalonia (NE Spain).

The driving force to develop this study has been the increasing need of prioritizing pollutants appointed by different water regulations such as the Regulation 166/2006 [1] concerning the establishment of a European Pollutant Release and Transfer Register. It aims at establishing a Community level register of integrated pollutant release and transfer (known as 'the European PRTR' or 'E-PRTR'). Its application domain affects certain types of manufacturing and production facilities, among them waste-water treatment plants (WWTPs) with a capacity of more than 100,000 equivalent inhabitants. Data gathered under the E-PRTR regulation provide a valuable source of information regarding the emission of pollutants to air, water and waste from the assessed installations.

The Catalan Water Agency (ACA) conducted three different campaigns with this purpose in 2008, 2009 and 2010. A total of 41 micropollutants belonging to different families (e.g. heavy metals, volatile organochlorine compounds (VOX), Polycyclic Aromatic Hydrocarbons (PAHs)), were analysed in the sampling campaigns and their potential risk for the environment and human health has been assessed using the here presented method. Appropriate referenced analytical methods were used to obtain concentration levels of these pollutants.

In order to ensure the appropriateness and consistence of this new method, the results have been compared with a well-trusted method used for chemicals prioritization, the COMMPS procedure developed by the Fraunhofer Institut [2] in 1999.

From the results gathered using the fuzzy model, it has been possible to characterize and compare the different sites (WWTPs) according to their associated risk as well as prioritize the compounds according to their relative risk. Finally, the fuzzy model has been compared with the COMMPS methodology, obtaining more conservative results with the first one due to the inclusion of persistence and variable's weight and the consideration of uncertainty. However, the behaviour of the two models is quite similar, fact that gives consistency to the fuzzy logic model.

TH 304

Simple equation allows assessment of biodegradation of cationic surfactants in activated sludge reactors (OECD 303)

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The Continuously-fed Activated Sludge (CAS) test is suitable to predict the removal of organic compounds from wastewater in activated sludge plants. However, a method to distinct between removal by adsorption and biodegradation is not provided in the Guidelines. Accurate knowledge on the relative role of the different mechanisms in the overall removal of surfactants in activated sludge systems is therefore not available.

The main operating parameters i.e. hydraulic retention time (HRT) and sludge retention time (SRT) are strictly maintained during operation of a CAS unit. The following equation to calculate the maximum concentration of surfactant adsorbed on the sludge was derived from a mass balance for the CAS unit:

$C_{surfactant\ sludge\ max} = SRT/HRT * (C_{surfactant\ influent} - C_{surfactant\ effluent})$. (Bio)degradation was assumed not to occur. This maximum concentration of surfactant absorbed on the sludge and the measured surfactant concentrations in the mixed liquid suspended solids allow calculation of biodegradation percentages. The removal of five cationic surfactants with varying adsorption capacities (decylamine, tetradecylamine, octadecylamine, dioctadecylmethylamine and dioctadecyldimethylammonium chloride) was assessed in CAS tests. The removal from the wastewater ranged from 98.8% (dioctadecylmethylamine) to >99.9% (decylamine). Removal by biodegradation was 69% (dioctadecyldimethylammonium salt) to >99.9% (decylamine). These removal percentages were compared with modelling results obtained with SimpleTreat. Simple Treat not only underestimates the potential of biological treatment systems to remove surfactants from wastewater but also the fraction removed by biodegradation. Especially, biodegradation of surfactants with a high potential to adsorb is not enough appreciated by SimpleTreat. The expression should be included in an update of the test guideline.

TH 305

Predictive modelling of steroid oestrogens in sewage effluent demonstrates the potential for endocrine disruptive effects in wild fish populations in South Australia

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Endocrine disruption (ED) has been observed in wild fish populations worldwide and is particularly well characterised in the UK, where the presence of oocytes in the male gonad and high levels of the female specific protein vitellogenin has demonstrated a feminisation response in wild roach (Rutilus rutilus). These phenotypes have been linked to sewage effluent containing the natural oestrogens 17β-oestradiol (E2) and oestrone (E1) as well the pharmaceutical 17α-ethinylestradiol (EE2) originating from human excretion. In contrast, little is known about the presence and effects of these chemicals in Australia, where there is a greater strain on water resources. Oestrogens have been measured in sewage effluents at levels comparable with the UK and there is some evidence of ED effects in fish downstream of sewage treatment works (STW's). It is therefore advisable to determine the potential levels of oestrogens entering Australian rivers and to assess the risk to wild fish populations. This study demonstrates the first use of predictive modelling of oestrogens in Australian STW effluent, creating predicted environmental concentrations (PEC's) for a set of STW's in South Australia and comparing them to the UK using a modified version of a model previously for risk assessment in both Europe and Japan. The PEC's were accumulated into an E2 equilivant concentration (EEQ) and were corroborated against measured concentrations from one UK and one Australian STW to determine their accuracy. These were then compared with the UK Environment Agency's predicted no-effect concentration (PNEC) of 1ng/L E2. Predictive modelling was shown to provide a good method for first tier risk assessment estimating the concentrations of oestrogens in effluents. The model overestimated the measured EEQ by an average factor of 1.4 when corroborated with the more robust UK STW data, however the effluent EEQ PEC's for all STW's were 3-31 times higher than the 1ng/L PNEC. The model demonstrated that although Australian STW's serve a lower population, their EEQ's are not significantly different to the UK's, which is likely to be due to the lower flow through Australian STW's which lowers the dilution factor. The results predict that effluents discharged in both the UK and Australia both contain oestrogens at concentrations exceeding the Environment Agency PNEC, suggesting that without sufficient dilution in rivers there is a risk of ED effects occurring in wild fish.

TH 306

Inputs of pollutants by urban wet and dry weather pollution in combined sewer systems: pau urban catchment (CDAPP, France)

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Urban inputs that include household effluents, drainage water, business effluents (e.g. car washes, dental uses, other enterprises, etc.), atmospheric deposition and traffic-related emissions (vehicle exhaust, brake linings, tires, asphalt wear, gasoline/oil leakage, etc.) transported either to wastewater treatment plants or directly to the aquatic environment, are expected to represent today important sources of pollution to the aquatic environment. Wastewater treatment plants represent a major threat to the aquatic environment since they are collecting a wide variety of sources from both domestic and industrial activities and they are continuously discharging various polluted effluents. Urban storm water runoff which is considered as transient source may also contain high levels of pollutants discharged directly in receiving streams by Combined Sewer Overflow (CSO). Implementation of the European Water Framework Directive and its affiliated directives requires Member States to improve their understanding of priority pollutants in urban areas and obviously within wastewater systems.

We studied the urban catchment of Pau urban area (south west France) which is about 50 km² with about 150 000 inhabitants. 4 rain gauges and about 40 flow meters were installed in all the study area to monitor the combined sewer network. Effluents were followed during about 10 campaigns including wet and dry periods for Suspended Solids (SS), Chemical Oxygen Demand (COD), Total Nitrogen (TN) and trace metals (Cd, Cu, Pb, Zn, Al, Fe, Cr, As, Sn). Emphasis is given to the characteristic, the partitioning and the contribution of pollutants from wastewater inputs during dry periods and wet periods in the combined sewer system of Pau. Concentrations of pollutants were combined with flow rate to assess fluxes that were directly discharge into receiving stream by CSO or carry on to the Waste Water Treatment Plant (WWTP). Following a storm event of about 2 hours we showed that this storm event induces an increase of fluxes by a factor of 1.5 to 5 compared to dry conditions. Half to two thirds of pollutants fluxes were treated by the WWTP during this period. The remaining part of the effluent was discharged in the receiving stream the river Gave de Pau. Fluxes of trace metals followed generally the same percentage than SS, suggesting that metal load is mainly associated to SS.

TH 307

Demography, economic activities and health equipments as potential sources of pharmaceutical compounds in wastewater and performance of wastewater treatment in two

French catchments
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Presence of pharmaceuticals in wastewater is attested by numerous studies. Although important research work is conducted on wastewater treatment plant (WWTP) performances, less is known on the contaminants sources. In fact, this information coupled with the knowledge of treatment efficiency could be very useful to manage WWTPs. In this perspective, the objectives of this work were to assess if pharmaceuticals concentrations in wastewaters could be explained by urban parameters in two urban catchments (A and B) and to compare their treatment performance.
Only 40 km separate both catchments, which have similar population density and distribution. However, catchment A has a higher number of inhabitants, health equipments and economic activities. Both WWTPs use the same technology (membrane bioreactor) to treat urban wastewater, but have different inflows (catchment A 10 times higher than B). Influents and effluents streams were monitored during 3 campaigns over 3 days with 24 hours composite samples. Pharmaceuticals and hormones were analyzed using LC-MS-MS. Results are shown for 6/32 studied compounds: acetaminophen (ACT), diclofenac (DIC), carbamazepine (CBZ), sulfamethoxazole (SMZ), iomeprol (IMP) and 17β-estradiol (E2).
Fluxes were 3 to 9 times higher in WWTP-A for ACT, E2, SMZ and DIC, 80 times higher for CBZ and 1350 times higher for IMP. The differences for CBZ and IMP were not only explained by the cities sizes but also by the presence of more potential sources in catchment A than B. Concentrations of DIC, SMZ and E2 were similar in both WWTPs. CBZ and IMP were respectively 10 and 183 times higher in WWTP-A. In contrast, ACT was 2 times higher in WWTP-B. Those concentrations could be explained by a dilution effect due to the higher discharge in WWTP-A. Then, in both WWTPs, concentrations of CBZ and DIC in effluents were respectively 0% and 6-42% lower than in influents. In contrast, IMP, ACT and E2 showed higher elimination between 76% and 99% in both WWTPs. Therefore, removal efficiencies SMZ was quite stable in WWTP-A (74% to 85 %) and greatly varied in WWTP-B (0% to 96%).
Results showed the presence of potential pharmaceuticals sources: some were strongly suspected, others needed further investigation. The main perspective could be to develop a methodology to predict the fluxes of compounds entering the WWTP based on urban characteristics to define suitable technologies for the best treatment efficiencies.

TH 308
Wastewater effluents and river waters: sources of organic micropollutants to NW Mediterranean coastal waters. Their characterization, fluxes and potential risks
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This study provides a first estimation on the risk associated with the inputs of organic micropollutants (OMPs) to coastal waters from NW Mediterranean Sea. Pollution sources evaluated were wastewater treatment plants (WWTP) effluents and river waters discharged to the sea. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorinated pesticides, polybrominated diphenyl ethers, phthalates and alkylphenols were analyzed by solid phase extraction and gas chromatography coupled to tandem mass spectrometry (SPE-GC-EI-MS/MS). Eight WWTP effluents and six rivers discharge an estimated amount of around of 25800 g d^{-1}. The concentration of ΣOMP in coastal areas ranged from 17.4 to 8442 ng L^{-1}. A summarized overview of the patterns and sources of OMPs contamination on the investigated coastal sea waters of NW Mediterranean Sea, as well as of their geographical distribution was obtained by Principal Component Analysis of the complete data set after its adequate pretreatment. Alkylphenols, bisphenol A and phthtalates were the main contributors to ΣOMP and produced a significant risk for fish, algae and the sensitive mysid shrimp species in seawater samples. The combination of GC-MS, chemometrics and risk analysis proves of use for a better management and control of OMPs discharges.

TH 309
Chemical characterization and treatment by enhanced coagulation of dissolved organic matter in biochemical-treated effluent of textile wastewater
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Performed with regular physicochemical-biochemical combined treatment process, textile wastewater effluent was treated by enhanced coagulation with a new kind of coagulant. Dissolved organic matter (DOM) in biochemical-treated effluent of textile wastewater as well as effluent treated by enhanced coagulation were isolated and enriched by extraction. Both DOM isolates were characterized by Gas Chromatography-Mass Spectrum (GC-MS) and both effluents by Excitation Emission Matrix (EEM). Results showed that biochemical-treated textile wastewater effluent mainly contained hydrophilic organic matters: triethylene glycol, tributyl phosphate and phthalates, and hydrophobic organic matters: amino acids and humic substances. And after enhanced coagulation treatment, most of the dissolved organic matter was removed from the biochemical-treated textile wastewater effluent.

TH 310
Emerging substances in aquatic phase of landfill leachate
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The potential environmental impacts related to landfill leachate are pollution of groundwater and surface waters. The risk of groundwater pollution is probably the most severe environmental impact from landfills because historically most landfills were built without engineered liners and leachate collection systems. Emerging pollutants are used in large quantities in everyday life and include a diverse group of myriad compounds (e.g., pharmaceutical compounds from different therapeutic classes, personal-care products (PCPs), flame retardants, industrial additives, anticorrosive compounds and others emerging species).The emerging substances are contaminants that have been recently discovered in the surface water due to their long-term, pseudo resistance and increased use in all industrial and human activities.Existing data show high leachate concentrations of all inorganic and organic compounds in the early acid phase due to strong decomposition and leaching. This paper presented the list of verified and detected EmS and various processes which take place in the aqueous layer of the landfill body as the typical heterogeneous system. It will be reported qualitative and quantitative data on the occurrence of emerging compounds with increasing environmental and public health concern. Products from chemical reactions and waste decomposition of emerging substances from aquatic phase of unsanitary landfills could contaminate the surface water, groundwater and aquifer. The preliminary results of Danube surface water in the vicinity of Novi Sad show presence of benzotriazole and caffeine. In some samples of groundwater the most frequently detected were diclofenac, ibuprofen, carbamazepine and metamizol metabolites 4-AAA and 4-FAA, within 19 pharmaceuticals that were analyzed in Serbia. This research was supported by Ministry of Education and Science, Republic of Serbia (Project number III46009 and 37014) and Program Science for Peace and Security (NATO), Project No ESP.EAP.SFP 984087.

TH 311
Removal of contaminants of emerging concern by a sewage lagoon
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Sewage lagoons are used widely in small communities in North America to treat municipal wastewater prior to discharge into surface waters. There has been little work to evaluate the efficacy of removal of contaminants of emerging concern by these small treatment systems. In this study, a sewage treatment lagoon for the village of Lakefield, ON in Canada was investigated to determine removals during the summer, fall and winter of selected PPCPs, including carbamazepine, trimethoprim, sulfamethoxazole, ibuprofen, gemfibrozil, triclosan, and HHCB and AHTN synthetic musks. POCIS and SPMD passive samplers were deployed at three locations in the sewage treatment lagoon, as well as at several points downstream of the wastewater discharge in the Otonabee River. Extracts from the POCIS and SPMDs were analyzed by LC-MS/MS or by GC-MS. Among the pharmaceutical target compounds, the highest concentrations in the sewage lagoon were measured in the fall for ibuprofen at an estimated concentration of 60 ng/L. Removal rates for ibuprofen and sulfamethoxazole were >75%, but carbamazepine was not removed effectively in the lagoon. Gemfibrozil showed a significant increase in concentrations as it passed through the sewage lagoon, which can be attributed to de-conjugation of metabolites during treatment. Trimethoprim was removed with intermediate efficiency (14-71%). There was a seasonal trend for removals of pharmaceuticals as they declined in the order of the sampling periods in the summer>fall>winter. Estimated concentrations of the target compounds in the river were very low (<1 ng/L) or below detection limits. The small scale sewage lagoon monitored in this study was effective at removing PPCPs at rates that were comparable to removals in conventional WWTPs.

TH 312
Removal efficiency of organic micropollutants in a constructed wetland assessed by passive sampling: results rom the WIPE project
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Despite advanced treatment techniques, STP's still are a major source of input of nutrients and micro pollutants into aquatic ecosystems. The WFD requires an ongoing emission reduction in order to meet its targets. On several locations in the Netherlands, wetlands are constructed, adjacent to the conventional STP. While retaining in the wetland, nutrients and pathogens concentrations decrease while oxygen content en biodiversity increase in the effluent. However, little is known about the fate of micro pollutants in the wetland. The aim of the WIPE project was to investigate if wetlands also contribute to a decrease of the environmental risk by removal of micropollutants from STP effluents. Therefore, we deployed passive samplers (silicon sheets) on 4 positions in 3 different constructed wetlands in 7 consecutive rounds from July 2009 till December 2010. Passive sampling enables the detection of very low concentrations of substances, because of their enhanced sampling volume compared to grab sampling. By its continuous sampling, passive samplers detect most substances passing the sampler, depending on the chemical properties of the compound and the sampling rate of the sampler. By this, a more detailed picture of the potential risk can be calculated. After deployment, passive samplers were analyzed for different groups of substances, and the risk of these substances was assessed using a Toxic Unit approach. In total around 250 substances were analysed on the sheets, and large differences existed between locations, but also between rounds. Surprisingly the largest group of substances, in terms of concentrations, were the pesticides, followed by flame retardants and personal care products (musks). Pharmaceuticals and estrogens only formed a small part of the total concentration. Hardly any removal of micropollutants was observed within the three constructed wetlands. In terms of risks large differences were observed between the locations, with one of the 3 locations having a risk approximately 2 magnitudes higher than the other

two locations. This was largely caused by a small number of pesticides with very low PNEC values being present at this location. Although not significant, all three wetlands showed a slight decrease in environmental risk from location 1 to 4. In terms of removal of organic micro pollutants, constructed wetlands only show a minor efficiency, and seem to be more efficient in biologically vitalizing the effluent.

TH 313

Assessing the potential of constructed wetlands in reducing environmental impact of STP effluents: the WIPE project

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At some pilot locations in the Netherlands, the effluent of sewage treatment plants (STPs) is lead through a constructed wetland (so called 'waterharmonica') before being discharged in surface water. This has been shown to revive the treated water, as oxygen demand is reduced and the biodiversity increases. The WIPE (Waterharmonica Improving Purification Effectiveness) project studied the potential of such wetlands to reduce the environmental impact of STP effluents on the receiving water, with special emphasis on ecotoxicological aspects. The (changes in) effluent quality of three STPs was monitored during the passage through constructed wetlands. This monitoring program consisted of passive sampling, chemical analyses, in-vivo and in-vitro bioassays, microbiology and biological and gene -expression responses of chronically exposed stickleback (Gasterosteus aculeatus). The monitoring was performed between 2008 and 2011 and generated an extensive dataset. In this presentation we will give an overview of how different environmental relevant end points were affected during the passage through the constructed wetlands.
In time large variations in the characteristics of the STP effluents were found. A wide range of chemicals was detected on the passive samplers, but in general these were not affected in a structural way by the passage through the wetlands. Occasional peaks in toxicity were observed, that indicate a potential risk for the receiving surface water. The intensity of these toxicity peaks was reduced after passage through the wetland. No indications were found that the effluents in general affect the reproductive success of the sticklebacks, although temporal poor effluent quality can affect survival. Gene expression indicated estrogenic effects on the male fish. This endocrine disruption was less at the end of the wetland where the effluent is about to be discharged on the surface water. This observation is in line with the results from the in-vitro-assays for estrogenic potential, and with the observation that the microbial community in the wetland sediments have a high potential to reduce estrogenic activity. Finally, it was found that densities of pathogens in the effluent were substantially reduced during passage through the wetlands. Constructed wetlands can thus help to improve the environmental quality of STP-effluents. Especially by levelling off high peaks in the toxicity and by reducing the density of pathogens.

TH 314

Inventory of emissions of priority hazardous substances in the surface waters in France

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Introduction

According to Article 5 of the Directive 2008/105/EC on Environmental Quality Standards in the Field of Water Policy, Member States have to establish an inventory of emissions, discharges and losses of all priority substances and pollutants listed in Part A of Annex I to this Directive. This paper aims at describing the french approach used to review of the impact of human activity on the status of surface waters through:
- the general working shem of the methodology and few definitions;
- the input data needed for the french approach;
- some exemples of calculations carried out on French river basins;
- the data compilation process.

Methodology guidelines

Methodology definition was guided by the following key ideas:
- A state-of-the-art methodology in agrement with reglementory requirments, and EU guidance.
- A non substance specific methodology. The approach is based on effluent discharges data; in their absence some explicit estimation formulas are proposed.
- A single methodology applicable at different spatial scales.
- A methodology that will work despite scarcity of information and data, and uncertainties.
- Future use of emission inventory as a management tool for designing srtategies for the reduction of the impacts of chemical pollution in watersheds.
- A methodology which can easily deals with data from different years. All available information which are representative of a given year to draw a coherent picture of the micro pollutants releases in surface waters during this year must be used.

After a bibliographical study, a pragmatic approach has also presided over the construction of the methodology developed in this paper in terms of work efficiency.
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TH 315

Corbicula fluminea: just an invasive species or also a freshwater bioremediator?

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Much attention has been drawn to the industrial and ecologic impacts of Corbicula fluminea, an invasive freshwater bivalve. This species has also been extensively studied as a sentinel for both potentially toxic metals and organic compounds. Such an attribute lies on the ability of these clams to bioaccumulate chemicals in body tissues together with a fairly large tolerance to the effects resulting from exposure. Additionally, the Asian clam seems to alternate filter-feeding at very high rates with pedal-feeding and deposit feeding, and hence an integrated view concerning different ecosystem compartments can be considered. As to our knowledge no attention has been devoted so far to the benefits that these features of the Asian clam may provide if applied to the treatment of contaminated waters.
The main aim of the present study was to generate preliminary results on the evaluation of C. fluminea bioremediation potential in the treatment of several effluents with industrial and commercial interest. This study is part of a broader assessment that considers a battery of wastewater effluents such as acid mine drainage, and pulp and oil mill effluents. The toxicity of both the initial clam-feeding solution and the post-filtration effluent to non target organisms (the bacteria Vibrio fischeri, the microalgae Pseudokirchneriella subcapitata and the cladoceran Daphnia sp.) was assessed through laboratorial bioassays. These bioassays were supported by the analytical characterisation of the exposure media before and after the clam filtration. Although the Asian clam is an invasive species and problems may arise from the intentional introduction in the discharge environments, controlled systems that allow us to capitalise on their filtration and bioaccumulation capacities may be assembled. Such a wastewater treatment method could bring huge benefits (e.g. output improvement and costs reduction) in the industrial context.

TH 316

Acute toxicity analysis of urban septic tank sludge

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The objective of this work was to evaluate possible toxic effects of sludge samples from urban septic tanks and upflow anaerobic sludge blanket (UASB) from a wastewater treatment plant located in the University of Espírito Santo, in Vitoria, Brazil. The method employed was the acute toxicity test Microtox (Azur Environmental) based on the bioluminescence inhibition of the marine photobacterium Vibrio fischeri. It is a test with many advantages such as ecological relevance, sensitivity, reproducibility, standardization and simple execution. A series of physical-chemical parameters were also analyzed based on Standard Methods for Examination of Water and Wastewater 20th (APHA - 1998). Sludge from four septic tanks was used in the study. From each tank four samples were collected, each aliquot representing a distinct depth, classified as: 1. scum; 2. liquid waste, 3. superficial sludge; 4. bottom sludge and 5. mixture from all the previous. From the UASB equivalent fractions were also sampled. The tests were executed according to the Microtox User's Guide Manual (AZUR Environmental, 1996) and results were expressed in EC50 (Effective Concentration), a value that represents the sample concentration in which 50% of the bacterial luminescence is inhibited. Only in one of the septic tanks, located in a public building toxic effects could be verified in the upper layers "scum"and "liquid waste". All samples from the bottom fractions presented higher toxic effects (EC50= 9.4%) when compared to the fractions above. The average EC50 for fraction 3 was 12.6% and 17% for the mixture, meaning even lower toxicity, possibly due to the influence of upper fractions that are stored in great volumes inside the tanks. The sludge from UASB presented higher toxic effects on topside (EC50= 5.5%) in comparison to the bottom (EC50= 8%), but lower toxicity when compared to tanks samples, since it was analyzed in a dilution of 50 parts in 200, while septic tank samples were diluted in one part in 200. All samples presented elevated levels of TKN, P and COD and were considered as stabilized sludge, since the relation VS/TS (volatile solids/total solids) was below 65%. Although many studies have applied the Vibrio fischeri test to a range of environmental samples, the analysis of sludge from septic tank represents a new approach, essential to evaluate the risk of releasing this residue in the environment or even ensure the quality required for its application in other activities.

TH 317

Comparing chemical analysis with literature studies to identify micropollutants to be treated or upstream source controlled in a catchment of Copenhagen (DK)

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The European Water Framework Directive aims at obtaining good ecological and chemical status of European water bodies by the year 2015 (EU, 2000). The directive also requires establishing pollution source inventories, to design and perform monitoring programs as well as to outline strategies to reduce emissions if the environmental quality standards are exceeded (EU, 2008). The aim of this study was to compare two different approaches to identify micropollutants in stormwater from a specified catchment; a literature inventory of potential pollution sources and chemical analysis of urban stormwater runoff. The selected catchment covers an area with roads, a shopping centre, a parking lot, office buildings, an upper secondary school and restaurants in Copenhagen (Denmark). The literature approach is limited to the range of included micropollutants and to how and which information is compiled, whereas the analytical chemical approach is limited to and affected by the sampling procedure, the selection of analysed

substances, available analysis techniques as well as analytical sensitivity and precision. Comparing the two approaches to identify contaminants reveals a discrepancy in the exact overlap of identical micropollutants, though the overall findings are similar.

TH 318

Toxicity identification evaluation for wastewater treatment plant working at different industrial and municipal scales

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A toxicity identification evaluation according international procedure was performed taking in consideration the system used for depuration of wastewater. Process control in the activated sludge plants were correlated with the toxicities of their liquid effluents. Mean cellular retention time (MCRT), hydraulic retention time (HRT), presence of anaerobic and anoxic selectors, sludge microbiology, and operation index such as Sludge Volume Index (SVI), diluted Sludge Volume Index (dSVI), sludge volume (Sv), base and spike Oxygen uptake rate (bOUR and sOUR), Mixed Liquor Volatile Suspend Solids (MLVSS), Mixed Liquor Volatile Suspend Solids (MLSS) and biofloculation index (Bi) were measured for each plant. A set of toxicity tests were carried out, together with the Solid Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis, for chemical characterization of each effluent. Data were interpreted by Principal Component Analysis (PCA) which allowed to classify for the first time, in Argentina, to rank in relation to type of industry, production volume and / or number of inhabitants. From the data interpretation arise that the high production volume chemicals (HPVC) are related with the higher download as toxic unit discharged into the aquatic environments of Buenos Aires province. It is need an urgent update of the parameters that govern the discharge of wastewater and the inclusion of their ecotoxicity assessment to allow the download.

TH 319

Application of delayed fluorescence to estimate the influence of waste water effluent on alga

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Waste water effluent is major source of chemicals discharge in to aquatic ecosystems. Since algae are important primarily producers in aquatic ecosystems, it is necessary to evaluate the influence of effluent not only on animals but also the algae. We are investigating a new method of for evaluating the influence of chemicals on alga by using delayed fluorescence (DF) from algae. The DF is a special type of luminescence; it detects the growth of only those cells that have photosynthetic capability. Since the DF originates from re-excitation of chlorophyll by a reverse reaction of photosynthetic electron transfer, the DF inhibition reflects inhibition of photosynthetic activity that is necessary for growth. Therefore DF is a potential endpoint for the estimation of the influence of chemical substance of algal growth in a shorter time than the conventional 72 hours growth inhibition test (e.g. OECD test guideline 201). The intensity of DF also shows a time decay curve. Chemical exposure changes the decay curve to patterns that have the potential to assist in the identification of contaminants in effluent sample. We have developed a rapid and simple DF test protocol using the green alga *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*). The test algae are prepared by thawing specially prepared -80°C frozen algae in a 1 hour pre-incubation. The prepared test algae can then be immediately exposed to the test sample prepared in a range of effluent concentration (0, 5, 10, 20, 40, 80 %) in small culture tubes (10 ml volume). The DF from algae in the culture tube is directly measured by the ultra-sensitive luminometer at 1 h, 4 h, and 24 h after exposure. DF is inhibited through the influence of the effluent. Further, effluent exposure changes the time decay curve of the DF intensity. From the relationship between the exposure concentration and the DF inhibition, we can estimate the dose-response of DF inhibition for each effluent or mixture. In addition, the pattern of the DF decay curve has potential for application to the identification of contaminants in the effluent. We will discuss results of a feasibility study evaluating treatments of industrial and agricultural effluents.

TH 320

Ecological health assessment and stressor identification using multi-response bioindicators of fish

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This study was evaluated the effect of environmental stressors using various parameters such as chemical water quality, habitat evaluation, and chemical exposure biomarkers in the Gap stream, South Korea during May-June 2009. We collected the pale chub (Zacco platypus) as sentinel species to evaluate biomarkers and indicators at four sampling sites in the Gap stream. The major changes observed were increased detoxification enzymes (EROD), DNA damage, physiological parameters, % of DELT anomalies, impaired reproduction, and reduced fish population structure in the downstream site (GS 7.2) of municipal wastewater treatment plant (MWTP). By the stressor identification (SI) process, five candidate causes of fish population degradation were winnowed including habitat alteration, low dissolved oxygen, nutrient enrichment, ammonia toxicity, and chemical pollution. The results of causal analysis suggest that the principal probable causes of fish population impairment in the downstream site below the MWTP were a combination of effects associated with recruitment failure and size-selective mortality caused by nutrient enrichment, ammonia toxicity, and habitat alteration such as degradation in spawning and nursery space.

TH 321

Monitoring potential ecotoxicological effects in the effluent of an urban wastewater treatment plant

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Organic micropollutants cannot completely be removed by traditional waste water treatment technologies. Therefore it is being discussed to add additional treatment steps such as ozonation, active coal filtration, UV-treatment etc. However, there is a lack of long-term studies to clearly prove a direct relationship between micropollutants in waste water and ecotoxicological effects on the macrozoobenthos fauna in the recipient stream to justify future xpensive installations . Online biomonitoring of the ecotoxicity of waste water effluents provides a quantitative relation between chemical composition of the waste water and fitness (behavior, survival, biomarkers) of the indicator species. The indicator species is chosen according to pollution-sensitivity, abundance and key-role in the stream ecosystem, in our case gammarids.
Gammarids were exposed in the Multispecies Freshwater Biomonitor[TRADEMARK] (MFB) placed in the UniPass[TRADEMARK] flow through system, receiving treated waste water just before release into the river Limmat. Potential short term responses to episodic pollution pulses as well as long-term effects of the potentially toxic cocktail of the waste water were monitored on real-time basis over several weeks. Different species of gammarids were applied (D. villosus, G. pulex) and their sensitivity compared. These studies establish the baseline toxicity of the waste water in its current treatment before additional treatment steps might be installed in the future in order to record the expected removal of the toxic potential. On the other hand, if during long-term monitoring with gammarids no toxic effects can be seen, combined with sporadic in situ benthos assessment, the need of additional treatment steps should be reevaluated on a case-by-case basis for each waste water treatment plant and its recipient river ecosystem.

TH 322

Implications of municipal wastewater on macroinvertebrate community structure and leaf litter breakdown

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European streams receive huge amounts of municipal wastewater, which, due to the limitations of conventional secondary treatment (i.e. mechanical and biological), may contain a wide range of micropollutants, like pharmaceuticals and personal care products. As such micropollutants are continuously released into aquatic environments, they may pose a potential risk for the integrity of ecosystems. Hence, the present study assessed adverse structural and functional implications of wastewater released by the municipal wastewater treatment plant (WWTP) in Landau, Germany, on its receiving stream, the Queich. For this purpose several endpoints related to the ecosystem function of leaf litter breakdown (microbial and invertebrate mediated leaf litter breakdown, in situ bioassays with *Gammarus fossarum*) and the structure of the aquatic invertebrate community were investigated for six and three weeks during winter and summer 2011, respectively. Macroinvertebrate related leaf litter breakdown in terms of leaf mass loss was by up to 65% significantly reduced at sites situated up to 500 m downstream of the WWTP outlet compared to the upstream site during both seasons. This effect may be explained by a meaningfully reduced abundance of leaf shredding macroinvertebrates such as *Gammarus*. Moreover, gammarids exposed in situ exhibited a significantly (up to 80% 100 m downstream during both seasons) reduced feeding rate at downstream sites, indicating lower energy availability and reduced reproductive output. These implications in leaf shredding macroinvertebrates and the ecosystem function of leaf litter breakdown may be on the one hand caused by direct ecotoxicological effects of the released wastewater and the containing micropollutants, which is supported by the results of the in situ bioassays. On the other hand, indirect pathways, driven by a lower nutritious quality of leaf material, indicated by a significantly reduced fungal biomass (up to 75%) at the downstream site, may have influenced the physiological fitness of shredders. Finally, the present study urges the evaluation of the current procedure in wastewater treatment by considering advanced treatment methods (e.g. ozonation), which may help to meet the requirements of the EU Water Framework Directive.

TH 323

Water quality investigations in the river Lea downstream a sewage treatment works: preliminary results of a case study

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In the north-east of London the Lea Navigation, a canalised reach of the river Lea, is affected by episodes of very low levels of dissolved oxygen. The problem was detected by the Environment Agency in the stretch from the confluence with Pymmes Brook (which receives the final effluent of Deephams sewage treatment works) to the Olympic area (Marshgate Lane, Stratford). In this study the river water ecotoxicity to the freshwater alga Pseudokirchneriella subcapitata was tested by algal growth inhibition test according to modified OECD guidelines (Organization for Economic Co-operation and Development, 2006). Results showed inhibited growth rate after 24 hours that was most marked when algal populations were exposed to the polar compounds fraction of the river water samples. Rapid acute toxicity tests were carried out using mediated amperometric whole cell biosensors (CellSense). Results indicated no evidence of acute toxicity in the river water, as no significant differences were detected between pre and post exposure responses to river water samples, with an exposure time of 30 minutes. These results indicate chronic pollution of the river, affecting photosynthetic algae during long-term exposure.

TH 324

Enrichment free LC-HRMS screening method of anthropogenic sewage pollutants in waste water, receiving water, ground water and drinking water samples

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In last decade, there has been growing public concern of potential contamination of water and environment with anthropogenic compounds and their degradation products and possible negative influence to the nature and public health. As a response to this fact, there is an increased interest in more efficient screening techniques of larger number of possible pollutants compared to that traditionally carried out by triple quadrupole mass spectrometers. The high resolution and accurate mass does not require optimization of compound specific parameters and has the ability to properly separate matrix from compounds of interest.
Fast and highly selective methods are necessary to screen, confirm and quantify different classes of contaminants in complex matrix and at low the analyte concentrations. High resolution mass spectrometry - using full scan analysis mode - has been described as the preferred screening tool due to the possibility to look for a large number of compounds and also because it enables retrospective analysis [1]. Furthermore, the use of a resolving power ≥ 50,000 FWHM is reported as being as selective as 2 SRM transitions when using triple quadrupole instruments [2].
In this work, the large volume direct injection has been applied to the screening of anthropogenic sewage pollutants in different matrices using a quadrupole-Orbitrap analyser. The experiment consisted on combining full scan mode at a resolving power of 70,000 FWHM with data dependent MS/MS spectra acquired at a resolving power of 17,500 FWHM. The MS/MS spectra were generated by using a high energy collision induced dissociation cell (HCD). This experiment was tested and evaluated in terms of quantification and confirmatory capabilities for the analyses of anthropogenic sewage pollutants. Different sample types were tested, including waste water, receiving water, ground water and drinking water representing different steps within the water cycle of densely populated and intensively agriculturally used areas.

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TH 325

Whole effluent assessment as an alternative to in situ ecological impact measurement? An experimental approach

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There is increasing recognition by regulators that there are limitations to the substance-specific approach for assessing and controlling the environmental fate and effects of effluents. Consequently, many regulators are seeking more holistic techniques such as whole effluent assessment (WEA) to supplement existing approaches. However, to ensure that these approaches are capable of indicating potential environmental effects, it is important to test scientifically robust WEA protocols. In general, WEA methodology assesses toxicity to aquatic organisms. WEA has relevance for the protection of ecosystems although the relevance and interpretation of results ultimately depends on the tests used.
In the context of the Water Framework Directive (WFD), European surface water should reach good ecological and chemical status in 2015. The ecological status is generally assessed with ecological indicators. In some particular cases (sampling difficulties because of access, type of substrate, several industrial waste in the area[3DOTS]), this approach is difficult to be applied. In this case, WEA methodology might represent an alternative to ecological indicators.
The main objective of the project presented in this paper, financed by TOTAL and the CONCAWE, is to show the real distance between WEA methodology which is conservative and in situ impact measurement for risk assessment. In this project, the difference between WEA and in situ impact measurement is assessed using dynamic outdoor mesocosms. This project has been designed and realized in three successive steps: (1) Preliminary experiments in Laboratory to assess the best solution to store one effluent in order to limit its variation (how to minimize volatilization, oxidation and crystallization how to resist to outdoor climatic variation[3DOTS]); (2) Feasibility experiments in mesocosms with one effluent (how to select effluent, to sample the effluent in industrial site, to transport, to store and to inject large volume of effluent; How to measure the ecological impact in mesocosms); (3) Final experiment in dynamic mesocosms with several effluents sampled in different sites and comparison with WEA methodology.
In this presentation the design of the three steps of this project will be presented and discussed.

TH 326

Multi-bioassay approach for assessing the impact of industrial discharges on the water quality in Wallonia, Belgium

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Bioassays offer an opportunity for a more holistic and meaningful way of assessing effects of environmental samples and wastes on ecosystems than what is possible by using chemical-based monitoring alone. They can provide predictions of environmental impacts whereas ecological community measures only determine impacts after they have occurred. Therefore, bioassays are useful in helping to implement the Water Framework Directive (WFD). In Wallonia (Belgium), an effect directed active monitoring using bioassays is being carried out for many years. It combines ecotoxicological measurements at emission and immission and physico-chemical measurements. We use a battery of short term and chronic bioassays with the bacteria Vibrio fischeri, the alga Pseudokirchneriella subcapitata, the rotifer Brachionus calyciflorus and the microcrustacea Daphnia magna. Moreover, a yeast estrogen screen (YES) assay was conducted as an assessment tool to detect the presence of endocrine disrupting compounds. During 2011, 14 major industrial discharges and 26 sampling points in the receiving waters (upstream and downstream of the effluent) were monitored. The effluents were sampled 6 times a year whereas receiving waters were sampled 4 times a year. Priority List substances of the WFD and other pollutants discharged in significant quantities were also measured. More detailed studies were carried out with complementary tools: upstream and downstream of a discharge of a metallurgical industry, bioassays were conducted on both water samples and sediments and bioaccumulation of metals was assessed using encaged bryophytes; upstream and downstream of a pharmaceutical industry, YES assay was conducted as potential assessment tools in combination with passive samplers (POCIS). The results of these monitorings show that bioassays are good diagnostic tools to determine the causes of poor ecological quality and to trace back to the source of contamination. They are an important “tool in the toolbox”for environmental management. They add value and provide complementary information to that supplied by the chemical and ecological community measures and could help to design appropriate management measures.

TH 327

Combination effects of pharmaceuticals and industrial chemicals in waste water effluents

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We will present our currently starting research activities in testing combination effects of pharmaceuticals and industrial chemicals known for their (co)-occurrence in WWTP effluents.
Mixtures of industrial chemicals and human pharmaceuticals can be found in surface waters as so called micropollutants. Effluents of waste water treatment plants (WWTP) are the main exposure route for both kinds of substances. The emission of these chemicals into WWTP originates from consumer usage of products, articles and drugs.
It is commonly known from literature and discussed in different scientific and regulatory communities that effects of chemical mixtures are significantly larger than single substance effects. At the moment neither in the environmental risk assessment of chemicals (REACH) nor of human pharmaceuticals (EMEA/CHMP/SWP/4447/00) possible combination effects in the environment is considered. But taking into account realistic exposure scenarios we assume that combination effects are the rule and not the exemption. Consequently an underestimation of environmental risks is likely.
The study focuses on the question if combination effects of pollutants in WWTP effluents require further assessment. Therefore ecotoxicity tests with algae and daphnids for 6 environmentally relevant substances shall be conducted individually and in different combinations respectively. These substances, (i.e. nonylphenol and ibuprofen) are known for their occurrence in WWTP effluents and their toxic effects to the aquatic compartment. Main objectives are (i) the quantification of the hypothesized underestimation of the risk by referring to single toxicity NOEC/EC10 values and (ii) the possibility to apply Concentration Addition (CA) as a default concept for the chosen scenario. The introduction of a potential mixture assessment factor (MAF) and its magnitude shall be discussed on the basis of the results gained.

TH 328

Illicit drugs as emerging pollutants in surface waters: acute toxicity test

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Today, illicit drugs are considered as emerging pollutants and several studies have been carried out to evaluate their concentrations in sewage waters and in effluent from WWTPs. Most of these compounds are not completely removed during treatment and are found in surface water at concentration around ten ng/L. Thus, it appears important to determine the ecotoxicological impact of illicit drugs.
In order to develop this knowledge, we carried out acute toxicity tests according to ISO11348. Four illicit drug families have been tested: cocaine, heroin, amphetamine-like and cannabis. In a first time, compounds were studied alone in tap water. Then, they were studied alone or associated in sewage water. Results will be presented and discussed according to the environmental concentrations of these compounds.

TH 329

Ecotoxicological characterisation of climbazole, an anti-dandruff agent contained in shampoo

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Effluents of municipal wastewater treatment plants are the most important sources for the entry of emerging pollutants like personal care products into the environment. Only recently, the anti-dandruff agent climbazole has been detected for the first time in the effluent of a German wastewater treatment plant with concentrations around 0.5 µg L-1. In raw wastewater and in activated sludge it was found with concentrations as high as 1.4 µg L'1 and 1.2 µg g'1 TSS, respectively. Climbazole is used as an anti-dandruff agent in hair care formulations acting as fungicide by inhibiting ergosterol biosynthesis. Despite its high efficacy against moulds and fungies, the possible impact of climbazole on the environment has only fairly been studied. The aim of this work was therefore to characterise the ecotoxicological effects of climbazole on aquatic and terrestrial organisms of different trophic levels. Assessing the terrestrial toxicity is particularly relevant when treated wastewater or sewage sludge is to be applied in irrigation or as organic fertilizer on arable land.
In single-species tests the toxicity of climbazole was determined towards the aquatic organisms water lentils, diatoms, green algae, fish embryos, daphnias and rotatoria and towards the terrestrial organisms bacteria, collembolas, enchytraeids and higher plants. All tested aquatic organisms were affected by climbazole concentrations below 15 mg L1 with the water lentil Lemna minor having the lowest EC50 in the µg per litre range. Among the terrestrial organisms the lowest EC50 was determined for the plants (Avena sativa and Brassica napus) with about 10 mg kg-1 soil dry weight for inhibition of shoot growth. Test results reveal that among the different trophic levels, climbazole is most toxic towards primary producers in the aquatic as well as in the terrestrial habitat. Displayed effects were a reduction of shoot length in higher plants and of leaf (frond) size in water lentil, which suggests an interference with the steroid metabolism of the plants. Assessing the terrestrial toxicity of climbazole is particularly relevant when treated wastewater or sewage sludge is to be applied in irrigation or as organic fertilizer on arable land.

TH 330
Environmental impact of current reserve-antibiotics with increasing incidence of severe infections using modern waste water treatment
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Antibiotic resistance is increasing since 30 years. Antibiotics previously used as reserve antibiotics, e.g. ciprofloxacine, are found in guidelines for treatment of slight infections. Due to uncritical prescription of broad spectrum antibiotics the amount of antibiotics used rises constantly. Many environmental studies proved the existence of different types of antibiotics in hospital sewage, municipal waste water, sewage disposal facilities and surface water or soil. For some substances critical concentrations with effects on algae, bacteria, daphnia and higher life forms were observed.
At the present Rifaximin, Piperracillline, Doripenem as well as the current reserve-antibiotics Tigecycline, Linezolide and Daptomycin are not classified as relevant to the environment because of their minor application. Hence environmental data for these substances are missing, as well as future schemes of use. Nowadays most environmental scientists are focusing on older frequently prescribed substances. As a result the risk assesement lags behind the modern pharmacotherapy and the achievments of the pharmacology.
Our objective is to study potential effects on the environment of above antibiotics. Ecotoxicological testing is performed in standardised bioassays using 24- and 96-well microplates for algae and bacterial growth inhibition tests. Ecotoxicological testing with algae growth inhibition test of new antibiotics showed increasing toxicity for Daptomycin and Tigecycline with EC_{50}-values of 14.4 mg/L and 1.76 mg/L. There is no toxic effect for Doripenem and Linezolid at maximum test concentration (EC_{50} > 100 mg/L).
Estimating PEC:PNEC-relations of these new substances has to take into consideration the increasing incidence of infections, change in demographics as well as biodegradability, compartment-distribution-tendencies and additional waste-water treatment techniques e.g. ultraviolett light, ozone and membrane filtration.

TH 331
Comparative study of the ecotoxicity of dishwasher detergents
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This study aimed at assessing the ecotoxicity of dishwasher detergents. Indeed, despite the high consumption of such products, available information regarding their environmental impact is still limited. Thus, twenty five of the most representative products available on the French market were selected, covering the different dishwasher detergent categories: "multibenefit"tablets, "classical"products (tablets and powder) and "gel"(both "classical"products and "gel"need the use of rinse aid).
Our approach was taken into account the ecotoxicity of solutions simulating a wash cycle in a dishwasher in typical use conditions, as recommended by the manufacturer. The ecotoxicity of these "washing solutions"was determined by performing a battery of aquatic bioassays from two trophic levels (algae and micro-invertebrates) covering both acute and chronic endpoints. In addition, the assessment of the ecotoxicity of two hand-dishwashing detergents, ten rinse aids (alone) and four dishwashing detergents (without addition of rinse aid in the "washing solution") was performed.
Both approach and selected battery of bioassays were relevant and allowed the classification of the different dishwasher detergents. Mostly, it was shown that the reproduction of C. dubia was the most sensitive endpoint compared with the inhibition of the mobility of D. magna and the growth of the algae P. subcapitata.
The "washing solutions"prepared with hand dishwashing detergents were found to be more toxic than those prepared with dishwasher detergents, except for the gel products. Regarding the automatic dishwasher products, it was shown that the ecotoxicity of "washing solutions"from the "gel"products was higher than the ecotoxicity recorded for "classical"products and "multibenefit"tablets. This study also revealed that the ecotoxicity of "classical"tablets was, most of the time, higher than the ecotoxicity of "multibenefit"tablets. Additional studies confirmed that the ecotoxicity recorded for "classical"products was mostly due to the addition of rinse aid in the "washing solution". These results suggested that the ecotoxicity recorded for ecolabelled products should be weighted regarding to the intrinsic ecotoxicity of the rinse aids which are currently not covered by "European Ecolabel".

TH 332
Characterisation of dissolved organic carbon in effluents and the assessment of its overall benefits in mitigating environmental impacts from metals
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Dissolved organic carbon (DOC) is known to form complexes with metals which can markedly reduce the free ion concentration of the metal and mitigate toxicity. Biotic Ligand Models (BLMs) for metals include speciation calculations of DOC with the metal ions, with increasing DOC concentrations usually providing a significant reduction in the bioavailability of the metal.
BLMs assume a composition for the DOC in terms of the ratio of humic and fulvic acids. These default DOC characteristics work well for natural waters with generally good agreement between predicted and observed no effect concentrations. Sewage effluent contains high concentrations of DOC but a significant fraction of this organic matter will not be humic and fulvic acids but proteins, other natural acids, chelating agents such as EDTA, sugars etc. which may bind metals more strongly than predicted by BLMs and so provide greater protection against toxic effects of metals. About 50% of effluent DOC has been shown to be relatively quickly degraded and therefore not likely to be humic or fulvic acids. The DOC in effluents will, therefore, almost certainly interact with metals in a different manner to that predicted by BLMs.
Also, any advanced treatment options used to destroy or remove organic chemicals of concern such as oestrogens or pharmaceuticals are likely to either reduce the concentrations of effluent DOC or affect its composition. Therefore, it may be that infrastructure investment intended to remove some organic chemicals may make the receiving waters more sensitive to metals by reducing protective effects of DOC.
This poster presents two practical elements designed to investigate the effects of advanced effluent treatment on DOC characterisation and metal speciation; the direct measurement of free metal ions in effluents over time using Donnan Membrane Technique (DMT) and Diffuse Gradient in Thin film (DGT) samplers, and the characterization of the DOC using UV spectrophotometry, Nuclear Magnetic Resonance (NMR), Size exclusion chromatography an pyrolysis GCMS - all carried out on the same sewage effluents.

TH 334
Integrating sewage treatment plant monitoring and modelling results to prioritise chemicals of concern and assess environmental risks
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Among the vast range of organic chemicals released via sewage treatment plant (STP), prioritising pollutants of concern and assessing their risks for the aquatic ecosystem entail key scientific challenges for regulators. From the EU perspective, such challenges are common to the chemical legislation, (e.g. REACH regulation, Biocidal Products Directive) and the water legislation (e.g. Urban Water Treatment and Water Framework Directives). In chemical risk assessment, the exposure of the aquatic ecosystem to organic chemcials released via STPs is calculated in two steps: first, local and regional scale emissions to sewage are estimated from consumer use data; then, the fate in a generic STP is calculated using the multimedia box model SimpleTreat. Default calculations are based on conservative (worst-case) assumptions.
A validation study was carried out for two chemicals of concern, LAS and triclosan, to evaluate the existing modelling framework applied to regulatory chemical risk assessment in the EU and to explore synergies with the monitoring and risk assessment activities under the Water Framework Directive.
Concentrations in raw sewage were estimated from product usage and chemical inclusion levels. The distribution and elimination in a conventional activated sludge sewage treatment plant with primary and secondary sedimendation was calculated with SimpleTreat 3.11, from the physicochemical properties and the biodegradation rate derived from STP simulation tests (OECD 303A). Probabilistic model simulations were run to account for the uncertainty of model inputs and the variability of STP parameters across the EU.
Calculated STP influent and effluent concentrations were in good agreement with measured concentrations collected from the literature and the probabilistic simulations captured, though not completely, the observed spread in observed values. Such modelling predictions can complement existing monitoring data and fill gaps for data-poor chemicals, thus supporting the prioritisation and the risk assessment of chemicals of concern. Moving from worst-case, default to more realistic, probabilistic exposure assessments facilitates the comparison of data generated and the harmonization of methods applied across regulations.

TH 335
Anaerobic biodegradation of PCBs in a grass cut batch reactor
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Due to properties such as high K_{ow} values chlorinated compounds including PCBs, dioxins or chlorinated pesticides in vegetation can lead to bioaccumulation in livestock and in the food chain. Hence, the remediation of these compounds in the environment is an important factor for human health. The degradation of the persistent Polychlorinated Biphenyls (PCBs) under anaerobic conditions in sediments and sewage sludge is described in the literature [1,2]. The potential for degradation in an anaerobic grass cut batch reactor has not been performed before and is investigated in this work. Therefore two series of batch experiments in laboratory scale (0.5 L to 1.5 L of volume) were performed under mesophilic conditions.
The first experimental series was performed at low natural, i.e. not spiked concentration level of Elbe riverside grass and sludge samples at 27 °C for 315 days. This first, explorative experiment showed ambiguous results for the degradation of the 6 indicator PCBs (PCB No.: 28, 52, 101, 138, 153, 180). Concentrations of the lower chlorinated PCBs decreased slightly, whereas the concentrations of the higher chlorinated PCBs were found to be stable at 5 20 µg/kg dry weight sewage sludge.
For the second experimental series 6 PCBs (No.: 28, 52, 101, 169, 138, 189), a technical mixture of PCBs (Aroclor 1260) and the pesticide Endrin were added in high concentration to the batch experiments (33 µg per compound, respectively and 100 µg of Aroclor 1260). In this second experiment different initial weights of grass cut from the Elbe riverside were mixed with anaerobic sewage sludge to determine optimal dry mass rates. For the determination of degradation rates experimental times for these batches vary between 30 and 120 days. Compound concentrations were measured by GC-ECD. Methane and carbon dioxide concentrations (measured by FTIR) in the gaseous phase of batch experiments were used as an indicator for biological activity. Results from the second batch experiment, concerning PCB-degradation rates and their correlation to gas production kinetics due to different dry masses, are discussed on the poster.

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TH 336
Removal of nutrients by immobilized microalgal beads in a continuous flow system
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Using immobilized microalgal beads in contaminant removal is an emergent method in recent environmental studies. As is well known, phytoplanktons use nitrate (N) and ortho-phosphate (P) during the photosynthesis process for production. In this study, a diatom "*Phaeodactylum tricornutum*"was immobilized in 3.5% sodium alginate solution and hardened with 4% calcium chloride (cation solution) and introduced into a continuous system to remove nitrate and ortho-phosphate. Daily samples were taken from the system and analysed by using UV-Vis spectrophotometer (Chebios Optimum-one) to determine the removal efficiency of the nutrients by the algal beads. The cell numbers in the beads were also determined by counting in a Coulter Counter system during the experimental period. The blank beads were also run in the system and subtracted from the total removal of the nutrients to detect the net removal by the algae. The system was operated as six separate runs with usage of the same algal beads by starvation of three days at each time to increase the removal efficiency of the system. It was observed that the algal beads remove approximately 50% and 40% (average values of net removal by the algae) of the P and N respectively, considering the inflow concentrations of 1000 mg/L of P and 3000 mg/L of N. Disadvantages of this method are indicated as the release of the microalgae out of the beads during the experiment and disintegration of the alginate beads over a long term run.

LC03P - Increasing scientific and policy understanding through meta-analysis of life cycle assessments

TH 339
Meta-analysis of diesel production in terms of life cycle GHG emissions and energy consumption: comparison and case study of Spanish refineries
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When performing the life cycle assessment (LCA) of petroleum products, life cycle inventory data are generally global compilations and they are not partitioned into sub-processes, but handled as a black box. Several products with different characteristics and uses are usually obtained from oil refining process (olefins, propane, butane, gasoline, kerosene, diesel, paraffin, asphalt, etc.). Allocation of proportional impacts of energy consumption and emissions for each product is a difficult task because many of them are correlated. Therefore, energy consumption and emissions are allocated according to the final product distribution. The most allocation methods used for petroleum products are often based on relationships of energy content, according to ISO standards.
This study compile the results of several studies of diesel production in European and American processing plants in terms of GHG emissions and energy consumption. Furthermore, this study is completed by recent studies of diesel production in Spanish refineries. Results highlight the broad range of values that depend on variables such as site-dependency or product specialization. Spanish refineries values are within the range of average European plants. Conclusions draw attention to the need of real data from refineries in order to obtain more adjusted and accurated results, as well as the possible partial subdivision of the system as a solution, according to ILCD handbook reccomendations for allocation procedures.

TH 340
Towards transparent and relevant use of energy use indicators in LCA studies of biofuels
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The use of energy has led to resource crises during the history of mankind, such as the deforestation of the Mediterranean during antiquity, and of Great Britain before the 19th century, and the oil crisis in the 20th century and continuing. Considering this, the frequent use of the impact category 'energy use' in the environmental assessment tool life cycle assessment (LCA) is not surprising. However, in a previous study, some of the authors noted that the term 'energy use' was not applied in a transparent and consistent way in LCA studies of biofuels. In this work we investigate how energy use indicators are applied in a set of life cycle assessment (LCA) studies of biofuels. In the examined reports and articles, the choice of indicator was seldom motivated or discussed and we observed five inherently different energy use indicators: (1) fossil energy, (2) secondary energy, (3) cumulative energy demand, (4) net energy balance, and (5) total extracted energy. These five energy use indicators were applied to the same cradle-to-gate production system of palm oil methyl ester (PME), giving considerably different output results. This is in itself not unexpected, but indicates the importance of clearly identifying, describing and motivating the choice of energy use indicator. All five indicators can all be useful in specific situations, depending on the goal and scope of the individual study, but the choice of indicators need to be better reported and motivated than what is generally done today. Authors of LCA studies should first define the purpose of their energy use indicator (fossil scarcity, energy scarcity, energy efficiency, cost/benefit comparison) and may then make a motivated choice of the energy use indicator.

TH 341
A meta analysis review: 'Drop-in' Biofuels - Life Cycle Assessments, substance flow analysis, material flow analysis and relevant information on an advanced biofuel
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The biofuel research and production sector is enjoying an unprecedented amount of public and private effort in order to remove various financial and technical barriers. One of these very important barriers is the challenge of developing biofuels that are compatible with existing gasoline, jet fuel and diesel infrastructure. The United States Department of Energy has been championing efforts that ensure that the next generation of biofuels will be regarded as "drop-in"biofuels. According to the Former Undersecretary of the United States Department of Energy, Kristina Johnson, "drop in"biofuels can be defined as fuels produced from various biomasses which are compatible with the over $9 trillion energy refinery and gas station infrastructure currently available in the United States.
According to definitions used in reviewed literature, drop-in biofuels are described by National Advanced Biofuel Consortium of the United States as infrastructure compatible - they can either be used directly or blended with their petroleum-derived counterparts. The European Commission on the other hand defines drop-in biofuels on the basis of quality specifications (standards) developed by the American Society for Testing and Materials.
Numerous LCA works have been conducted concerning what is described as drop-in biofuels. Although this topic of drop-in biofuels is relatively new, literatures regarding LCA and drop-in biofuels based on the definitions provided were available for analysis. A comprehensive study was undertaken with the aim of providing a clear overview of the previously conducted LCA studies in this new field of advanced biofuels. Also, important topics concerning this new chapter in the biofuels revolution were delved into. The results of this meta-analysis review will be presented at the SETAC world congress.

TH 342
How to assess the data quality of LCI studies - a systematic approach
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The field of energy from biomass is rapidly growing, increasing the need for reliable data on the environmental burdens inflicted by the use of the different conversion technologies. A huge number of LCI studies on these topics can be found, however, most of these studies are not directly comparable to each other and their results may vary considerably depending on different data origins, time and geographical background, technologies and system boundaries.
Therefore it is often unclear how to decide which study is "best"suited for a given case.
The objective of this work is to present a guideline that helps compare different LCI studies. In this guideline a systematic approach on the assessment of literature quality is done. In a first step the current "best practice"for modelling an LCI is summarized. By this a reference for the assessment of the data is defined. This reference will identify a

set of indicators.
In a second step on the base of the indicators developed in the first step the available studies will be analyzed. In the last step, the actual states of the data are compared and assessed with the reference. This approach makes it easy to spot and report any shortcomings the studies may have and eventually decide which study should be used for a given problem.
The guideline consists of four parts:
- Description of the technology of interest and of available literature
- Description of the indicators, the reference values and the available data
- Evaluation of the available literature
- Recommendation
The guideline was tested on an analysis of LCI studies and data sets of an Organic Rankine Cycle plant for German framework conditions.
Using the guidelines data gaps were identified and quantified resulting in a better overall understanding where these gaps came from and how to deal with them. The guidelines allowed for a good comparison on the data quality of the literature. With their use a good overview of the different LCI studies could be achieved and a clear recommendation for data set generation could be given.

TH 343
Finding LCA research direction with the aid of meta-analysis
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Rapid growth in LCA methodological developments has generated a large body of work in the last ten years. New impact categories, indicators and characterization factors have been developed with regard to the LCIA. Moreover, an increased sophistication of the existing methods of characterization has been also proposed, for example with the introduction of spatial and temporal differentiation. On the side of the inventory analysis, the need to assess policies, technological and structural choices characterized by scarce reversibility, long term effects, trade-off between environment, economy and society, etc. has been driving an expansion of the scope of LCA, both in terms of level of analysis (from products to systems, from micro to macro) and coverage of indicators (including also economic and social aspects). Consequential LCA, hybrid approaches combining LCA and input-output analysis, scenario modeling, and new efforts for developing social and economic assessment methods, are only some examples of the developments LCA methodology has been going through. Overall, it may appear that LCA lacks direction on how to further develop.
In this paper the authors developed and applied a structured approach, inspired by the meta-analysis concept, to examine literature and identify research thrusts on how to further develop LCA. The procedure consists of four-steps: i) definition of the research question; ii) carrying out a literature review concerning more than 280 papers - selected from about 2000 articles according to pre-defined criteria - which resulted in the identification of some 60 main methodological topics; iii) research gap analysis, in which the methodological topics identified in the previous step were compared with the research priorities identified through a users' needs survey; iv) interpretation of results, in which the results of both the previous steps were evaluated and organized into coherent research thrusts.
Overall the analysis delivered two main research thrusts: one devoted to increase practicability of LCA, the other to increase model fidelity. The former is aimed at making knowledge available in easily usable way, while the latter focuses on better describing the complexity of the systems analysed and those interrelations that are really meaningful. Specific research topics were identified for each thrust, which suggests that sophistication and practicability can and should coexist in the same method.

TH 344
A web-based approach to handling divergence in LCA
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The last two decades have seen a profusion of studies in the Life Cycle Assessment (LCA) field, as well as growing interest in this work by parties in business and government. Furthermore, the rapid growth of detailed and constantly updated Life Cycle Inventory (LCI) and Environmentally Extended Input Output (EEIO) databases has led to an impressive set of studies and published information, from which it is often very difficult to elicit conclusive assessments due to conflicting results presented by the authors, although ostensibly the same product is being analysed.
At present, a widely discussed approach to handling this situation is through some form of meta-analysis. However, we think it is useful to explore other ideas and approaches, in particular, those that afford solutions that may be available sooner, and also available across a wider range of cases and for a wider range of users.
Our general strategy is to take a more qualitative approach to the problem of divergence or discrepancy in LCA. We think about a web-based toolkit that tries to resolve discrepancies by opening up the information using distributed data. An option for accessing distributed data sources and linking them semantically is the emerging technology of Linked Data (LD). LD technology aims at allowing meaningful interlinks between all sorts of resources within the World Wide Web. It can be seen as a further development of the usage of hyperlinks, extending the simple link (which can be seen as the sentence 'has to do with') to meaningful relations (such as 'isObservedOn' [observedEntity]). While the primary units of the hypertext Web are HTML (HyperText Markup Language) documents connected by hyperlinks, Linked Data relies on documents containing data in RDF (Resource Description Framework) format and residing in the *Web of Data*.
Essentially, our web-based toolkit is supported by a Data Access Layer which will provide search capability over LCA information available in RDF format. We envision that a form-based search interface will allow a user to specify details about the desired quality criteria and on a specific subject and run a *structured query* over the *Web of Data*. In this way the divergencies will be directly resolved at the level of the Data Access Layer.The (free-to-use) basic system we envision will support simple assessments and will help LCA users to produce more robust and policy-relevant results.

TH 345
Environmental impacts of palm oil biodiesel: a Meta analysis
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Environmental impacts associated with the life cycle of palm oil biodiesel is reviewed in this study using meta-analitic method. Most studies found palm oil biodiesel would produce positive energy balance with an average net energy ratio of 3.27, which exhibit the strong potential of palm oil biodiesel as renewable fuel. However, it was found that palm oil biodiesel is a net emitter of GHG to the atmosphere. The origin of oil palm plantation is the foremost determinant of GHG emissions. Converting peatland forest results in GHG emissions up to 60 t CO2eq ha-1 yr-1. In contrast, converting degraded land or grassland for plantation can positively offset the system to become a net sequester of 5 CO2eq ha-1 yr-1. Some other cradle-to-grave environmental impacts associated with palm oil biodiesel are acidification, eutrophication, toxicity and biodiversity.

TH 346
Greenhouse gas emissions from wind power: a critical meta-analysis
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In this paper a range of historic lifecycle studies of greenhouse gas-equivalent emissions for on and offshore wind farms are reviewed to identify a definitive set of the most current, original, and transparent studies. It begins by briefly detailing the separate components of the wind farm life cycle before explaining the methodology of the meta-analysis and exploring the range of lifecycle estimates. The paper highlights that ranges of emissions estimates are present for wind power. The paper also suggests that meta-analyses of historic lifecycle studies of this nature help to provide greater advice ensuring comparability, consistency and accuracy for given technologies. The paper then explains some of the factors responsible for the disparity in lifecycle estimates, in particular identifying errors in both the lowest estimates and the highest estimates. It is noted that wind power is not directly emitting greenhouse gas emissions, but rather that lifecycle emissions occur through farm construction, operation and farm decommissioning.

TH 347
The renewable energy directive and cereal residues
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The Renewable Energy Directive provides a methodological framework for a streamlined LCA that calculates the greenhouse gas (GHG) balance of biofuels in order to determine whether they reach the required 35% GHG reduction threshold. It describes which sources of emissions should be included and describes how co-products and residues or wastes should be allocated, however a clear definition of these is lacking. Currently, in order to incentivise biofuel production from second generation sources such as cereal residues and wastes, double credits are to be awarded to these biofuels, and the feedstocks are considered to be available at zero GHG 'cost'. This presentation will consider if cereal residues removal should be accounted for in GHG reporting for biofuels, as there are potentially sustainability implications with removing residues from agricultural land or from markets, and combusting them. The RED does not consider these potential impacts, which questions the ability of streamlined LCA's to account for the sustainability of these biomass resources. This presentation will compare results from streamlined LCA's and a full environmental impact assessment of cereal residue removal and combustion. The results will indicate the importance of inclusion of direct and indirect LCA impacts in policy analysis.

TH 348
Meta-Analysis of LCA studies for bio-based polymers: assessing the environmental performance of polyhydroxyalcanoates (PHA) and polylactide (PLA) in contrast to their petroleum-based counterparts
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The depletion of non-renewable resources plays an important role in present political debates: On the one hand, the depletion of non-renewable resources results in increasing commodity prices and economic dependence. On the other hand, the emission of greenhouse gases causes severe effects on the climate with unpredictable impacts on the human environment. That is why energy from renewable resources as well as bio-based chemicals and materials have attracted great interest.

Polyhydroxyalcanoates (PHA) and polylactide (PLA) are appropriate bio-based substitutes for a variety of previously petroleum-based products. Life Cycle Assessments (LCAs) according to ISO 14040 and 14044 have been applied to compare the environmental performance of these bio-based polymers in contrast to their petroleum-based counterparts. Numerous cradle-to-gate studies have been carried out to analyse the environmental impacts of the production of polymer resins with a preliminary focus on the impact categories climate change and non-renewable energy use.
Previous studies indicate a wide range of results and contradictory conclusions due to the consideration of different production processes, methodological choices (e.g. system boundaries, co-product allocation, impact assessment methods) and underlying assumptions. To produce more robust and policy-relevant results than induvidual LCAs, an approach for a Meta-Analysis is developed and undertaken to analyse the outcomes of 25 LCA studies. The underlying studies are quantitatively assessed taking into account the impact categories climate change (measured in metric tons of carbon dioxide equivalents) and non-renewable energy use (measured in megajoules). Relative environmental impacts per metric ton of PHA and PLA are compared with corresponding values for the petroleum-based plastics polypropylene (PP), polyethylene (PE), polyethyleneterephthalat (PET) and polystyrene (PS). Furthermore, differences between the environmental impacts of bio-based and petroleum-based polymers as well as standard deviations are calculated to derive methodological consistent figures.
Results of the Meta-Analysis indicate environmental advantages and disadvantages in both impact categories depending of the selected pair for comparison between bio-based polymers (PHA, PLA) and petroleum-based polymers (PP, PE, PET, PS). Furthermore, the Meta-Analysis reveals that bio-based polymers can help to mitigate climate change and save non-renewable energy.

TH 349
The effect of system boundary and weight of emission factors along the life cycle on the results of a life cycle assessment (LCA) - Electricity generations as example systems
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LCA is a widely used tool for determination of strength and weaknesses regarding ecological aspects of any product or service. It is expected from the scientific-based analysis to deliver robust information, but in fact in literature the results differs significantly for the same product or services. Due to the recognized uncertainty this study addresses the possible deviance of results from literature for electric energy production from different energy sources. The potential deviation could arise from the consideration of different system boundaries (SBs) and the relevance of emission sources along the life cycle. Both could be mainly responsible for the recognized diversity beside others like allocation, cut-off criteria, actuality of literature, etc.
The electric power generation gives a good instance to analyze the impact on the results of the different SBs. For example: the variation between different plant construction demands is often over 80% , but the overall results don´t necessary correlate with this deviation. The change of cement demand for construction by 230%, in the case of electricity generation from coal, induces only 1.7-2% difference in GWP of the total system. This is an effect of different proportion of emission sources between the life cycle stages.
The contribution will point out where uncertainties and dependency of the results could arise through the examples of electricity generation from coal(lignite), natural gas, hydro energy, nuclear energy and from wind energy. The whole life cycle of power plant (with fuel upstream), the following system parts and impact categories have been taken into account by the understanding the results of LCAs:
- Construction, fuel upstream, transport, production, operation, decommissioning as life cycle phases
- Construction demand variables (basic construction materials: cement, steel, etc.)
- Fuel demand variables (efficiency factors: fuel purity, thermal efficiency, wind yield, transport, etc.)
- Impact assessment methods (GWP as main LCIA method, eutrophication and acidification potentials)
The chosen system boundaries and other variables contribute to the varying results of LCA studies for electric energy production. Data from the literature should only be used from reliable sources and after thoroughly investigation, if the SBs and information of the used variables is well described. Otherwise the unreflected use of a data source might lead to false results and recommendations.

LC06P - Life cycle management (LCM): Success factors and barriers

TH 350
Using LCA in the preparation of the new waste management plan of Regione Lombardia
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Regione Lombardia has among its tasks the development of management strategies for municipal waste produced in its territory, in collaboration with provincial governments, the control bodies and public and private entities that operate on the collection, recovery and disposal cycle.

In order to pursue sustainability also in this field, Regione Lombardia has decided to consider “environmental performance”as a criterion for supporting future planning decisions, and therefore stressed the need for specific assessments of environmental impacts associated with the planning in the waste field.
Life cycle assessment (LCA) has thus been chosen by Regione Lombardia as a strategic support decision tool in the preparation of its new waste management plan. The goal is to use the life cycle thinking approach to assess the current regional situation and thus to give useful strategic indications for the future waste management. The project (called GERLA: GEstione Rifiuti in Lombardia - Analisi del ciclo di vita) started in November 2010 and will be completed by the end of 2011.
The first phase of the study consisted in the analysis of the present management of municipal waste in Lombardia Region (baseline scenario - reference year 2009). This has involved the identification of all the fluxes of materials (both the source-separated fractions and the unsorted residual waste) in terms of quantity, composition and destination, together with the characterisation of the most important treatment plants, in terms of their capacity, energy and materials consumption, emissions in the environment, energy and materials recovery. After that, the LCA methodology was applied to evaluate the energetic and environmental impacts associated with this baseline scenario.
The critical analysis of the results (Life Cycle Interpretation) of the baseline scenario has allowed the definition of a number of future scenarios for the year 2020, with the final goal of improving the environmental performances of the regional system.
This study represents thus an important application of LCA methodology since, for the first time in Italy, the outcomes of a detailed LCA of a current waste management system are actually utilised in the waste management planning on a regional scale.

TH 351
Life Cycle Assessment for end of life computers in Mexico
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The recovery of waste electrical and electronic equipment (WEEE) is an activity that becomes more important every day in Mexico. In 2010 electronic waste generation reached 307.000 tons annually. Of these, 10% is recycled or valorized, el40% remains stored in houses and 50% is sent to final disposal in landfills and open dumps. The aim of this project was to conduct a life cycle analysis using the program Umberto for electronic waste management in Mexico and compared its environmental impacts with those for three proposals for change in the end of life management pattern in order to determine the best option for a national policy. The policy proposals were modeled as follows: 1) Stage with 25% of recovery system , 2) Stage 35% to recovery system, and 3) 0% for disposal in open dumps. As conclusion it was identified to eliminate the use of open dumps for waste disposal in first place, followed by an increase in 35% of the recycling/valorization system.

TH 352
Implementation of an integrated technological-LCA modelling tool within the water industry - a pragmatic contribution to decision-making
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Nowadays, the water industry is expected to better take into consideration environmental issues. But it still has to deal with operating costs, sanitary risks, technical feasibility and reliability of designed plants. Therefore, the water industry managers need handy tools and practical methodologies that can provide them a complete set of information for projects under study. A good implementation of such decision supporting tool is obviously a condition to its success.
Within the EVALEAU research project, an integrated technological-Life Cycle Assessment modelling tool has been developped with the software Umberto®. The tool is based on an exhaustive library of unit process models. These models are highly detailed and parameterized, thus precisely describing the energy and mass balances depending on a specific project context. Complementary software tools are directly linked to the tool, which makes it benefit from previous modelling efforts and specific industry knowledge. For example, the tool is linked to the software PHREEQC® which simulates chemical reaction.
Every unit process model generates an engineering design report when placed within a modelling scenario. These reports are spreadsheets containing engineering design data (e.g. pumps power). This information is interesting at the design stage since decision can be made knowing how the process will work on the field. Problematic technological solutions can be avoided whereas convenient technologies will be selected.
A code for sensitivity analysis of the process model parameters is also part of the framework of the tool. The mathematical method followed is the Morris method. It aims at detecting the key parameters of the modelled scenario by qualitatively estimating their influence on any of the results (e.g. one selected environmental impact or the operating cost). Decision-makers can better understand how the modelling scenario react to a change on one model parameter. Assuming that the modelling scenario is realistic enough, conclusions can be made on the real plant. This key feature of the tool gives the designers good clues about the future plant functioning and indicates them which are the priority action levers.
The success of the tool implementation relies on its convenience on the field, obtained by previously developping it closely with its future users. Not only environmental issues are taken into account but the tool give the whole picture of an industrial project.

TH 353
LCA on drinking water production from pesticide contaminated ground water
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This poster presents the results from an LCA case study addressing a growing Danish problem on pesticide contaminated ground water. The conventional Danish way of using pesticide free groundwater and treating it by aeration and sand filtration, is compared to using slightly pesticide contaminated groundwater and adding treatment by activated carbon filtration and UV-treatment. The scenario of using slightly pesticide contaminated groundwater is chosen as it is a genuine and relevant alternative in the current Danish situation as groundwater wells are closing due to pesticide contamination and clean ground water is becoming scarcer. Besides the comparison, a hotspot analysis is performed in order to identify the dominating environmental impacts in the life cycle of generating drinking water from ground water. This study is part of the research project DWBiofilters (www.dwbiofilters.dk) aiming at improving sand filters for drinking water production by investigating its inherent microbial processes.

TH 354
Development of a new LCA tool for maintenance at the initiative of the users - a bottom-up approach
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Many practical LCA tools have been developed before a user group was established. The risk of such a top-down approach is a gap between the tool and the way users want to use the tool. This mismatch may result in a tool being less used by the users. In the Netherlands, a group of housing associations and building and maintenance companies were looking for means to calculate the environmental performance of planned preventive maintenance of housing, estates and buildings themselves. There are several theoretical approaches available, but no practical calculation tools. Because of this lack of instruments, they set up a development project for a practical calculation tool, to be developed by the consultancy company W/E and by the Delft University of Technology, who have much experience with LCA in construction. In the development project, generic environmental data on materials were combined with data of maintenance activities gathered by the maintenance companies. The set-up and interface of the calculation tool were designed according to the wishes and practices of the clients. The results are presented according to the three purposes of the tool: optimisation of the environmental performance of maintenance scenarios, comparison of different scenarios, and benchmarking the scenarios with average Dutch maintenance scenarios. The resulting calculation tool is a good example of the effectiveness of this bottom-up approach, aiding to achieve a broad support amongst potential users of this tool.

TH 355
Improving Life Cycle Management (LCM) tools for the food industry: a framework of Product-Oriented Environmental Management System (POEMS)
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The aim of this abstract is to present a LCM tool designed for the agri-food industry, a POEMS framework with a modular structure resulting from the integration of complementary tools: an Integrated Management System (IMS), a simplified Life Cycle Assessment (LCA) and a suitable Environmental Product Label or Declaration (EPLD) (EMAF Project co-funded by the Italian Ministry of Education, University and Research-PRIN 2008TXFBY). In order to successfully apply this framework in the agri-food firms the main sector-specific barriers (SSB) to LCM implementation have been used as starting points to set the success factors (SF), solutions (S) and tools (T) of the POEMS model. In the following the path to the POEMS framework definition is summarizes: the path efficiency is enhanced by the fact that each tool can provide multiple solutions to several barriers.

SSB: resistance to change; dispersion of the environment-related information; in Envtl. Management Systems (EMS) little attention is paid to product performances **[ARROWRIGHT]SF:** spreading an envtl. cultural change and involvement; structural and organized vision of envtl. aspects; internalize product requirements within the EMS **[ARROWRIGHT]S:** envtl. training and dissemination; EMS; Integrating EMS with a Quality Management System **[ARROWRIGHT]T:** Integrated Quality and Envtl. Management System

SSB: main focus on short-term problems; lack of chain management responsibility; lack of awareness of product life cycle envtl. impacts (PLEI); poor access to large amount of life cycle data; lack of in-house expertise; high expensive **[ARROWRIGHT]SF:** internalization of chain management vision and long-term value creation; identification of PLEI; allow SMEs to perform envtl. assessment by themselves providing an easily understandable tool **[ARROWRIGHT]S:** LCM and Life Cycle Thinking; LCA; simplified approaches **[ARROWRIGHT]T:** Simplified LCA

SSB: envtl. commitment is not perceived as an opportunity; problems with results communication and chain involvement; complexity and uncertainty in choosing the most suitable envtl. message **[ARROWRIGHT]SF:** ability to transform the envtl. measures taken into commercial advantages; spreading envtl. cultural change and involvement with external dissemination; identification of the proper envtl. label/declaration **[ARROWRIGHT]S:** EPLDs; envtl. product communication; guidelines to support decision making **[ARROWRIGHT]T:** EPLD guidelines

IMS+Simplified LCA+EPLD guidelines=POEMS
The EMAF project is in progress with applications in the canned vegetables, wine, pasta, olive oil and coffee supply chains.

TH 356
Finnish guidelines on carbon footprinting - supporting practical implementation of LCA in the food industry
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Life cycle thinking is spreading among environmentally responsible companies around the world. Specially carbon footprint guidelines, which have been developed in the last years, have increased the interest of industries to life cycle assessment on company and product level. Growing general knowledge and pressure to act on climate change has given incentives to companies to use carbon footprinting in their consumer communication as well. Consumers show growing interest to, for example, carbon footprints in many Gallup polls.
Many international standards and guidelines on LCA are published but no standard widely approved method on evaluating environmental impacts of food are available. In the Foodprint - research programme sound national methodology for calculating carbon and other footprints of food has been developed in close collaboration with the Finnish food sector. As published guidelines are too generic to give practical instructions to companies to produce comparable carbon footprints for communication, the project has specified detailed methodology to follow. International standardisation, developments and best practices on evaluating environmental impacts are taken into account when preparing national specific guidelines. Finnish food sector is actively consulted to ensure practical methodology which can be easily applied.
In addition to development of national calculation guidelines, the project is also providing practical calculation tools to facilitate calculations at company level. The project also tested different data collection tools in R&D projects.
The project has been carried out as iterative process between research, companies and other stakeholders. Food industry wide workshops around the guidelines are used to make the guideline more applicable and user friendly for the industry. As many Finnish food companies use carbon footprinting, industry participation is high. The guidelines were also reviewed in a detailed way by other Finnish LCA experts from LCA and from other standards' point of view.
The company R&D projects were crucial for developing practical guidelines for the industry. Motivation of the whole supply chain of the companies in the beginning of the project is fundamental. Additional workshops for the whole industry gave important feedback and shall facilitate larger implementation of the guidelines.

TH 357
Life cycle thinking applied to an immunological product (vaccine) used for boar taint control in male pigs
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In 2009, Pfizer Animal Health (USA) started to apply the Life Cycle Assessment (LCA) methodology to some innovative products, with a first case-study on ImprovacTM, an immunological product (vaccine) for male pigs that, by providing farmers with an alternative way to avoid the problem of boar taint, also allows them to increase the efficiency of male pig production, which may in turn provide considerable life-cycle environmental and social aspects benefits: to avoid physical castration is in fact perceived as an animal welfare issue by many in the public sector.
In 2010, after an initial project which included a consistent data collection from the vaccine production plants as well as from farms and slaughterhouses at global level, the first Environmental Product Declaration or EPD on a veterinary product was published by the International EPD System, a well recognised ISO 14025 Program Operator (www.environdec.com); the results of this project were presented for the first time at the 2011 Annual European SETAC Conference in Milan.
In late 2011, the sample of farms participating in the global survey was extended for the development of the renewed and updated EPD to be published by the end of January 2012.The new data collection work led to the inclusion of a considerable number of representative farms from other countries worldwide.
This analysis provided LCA data to assess possible environmental benefits of the vaccine's application: the reduction of the carbon footprint is a strong perceived advantage for the agricultural and food sectors and for this reason, it is the major impact category of interest; other LCA relevant impact categories are presented and discussed to provide a comprehensive view of the life-cycle impacts of the product. The calculated carbon footprint for the Improvac pig system demonstrates a reduction vs. the physically castrated pig system, mostly depending on country specific feed production and slurry management practices. Starting from the feed components, a detailed examination was conducted on agricultural practices by country; the same holds for different slurry management procedures and technologies.
For this reason, the information collected and analyzed in the LCA process provides an important opportunity for swine producers to understand the magnitude of their

carbon footprint and how to potentially reduce it not only through the use of Improvac.

TH 358
Pilot test in food sector of product environmental footprint (PEF) Guide developed by European Commission
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In 2011, the Directorate General for the Environment (DG ENV) started to work with the Commission's Joint Research Centre (JRC) Institute for Environment and Sustainability (IES) and other European Commission services towards the development of a harmonised methodology for the calculation of the environmental footprint of products.
The Product Environmental Footprint (PEF) Guide developed by JRC IES has been tested using a limited number of pilot studies representative of a wide variety of goods and services. Pilot tests have been conducted on five Nestlé food and beverage product systems: Nespresso, Purina Gourmet Pearl Chicken and Vittel products have been quantitatively assessed whereas Nescafé and Kit Kat products have been qualitatively assessed.
The results of Nespresso case study according to PEF Guide are presented and they are compared to the results of the full life cycle assessment compliant with ISO 14040 and ISO 14044 published in 2011. The comparison highlights some differences related to specific modelling issues as Life Cycle Inventory (LCI) choices (e.g. electricity mix), LCI databases (e.g. ecoinvent or European Reference Life Cycle Database (ELCD)) and Life Cycle Impact Assessment (LCIA) methods (e.g. land use, terrestrial ecotox and turbined water as well as endpoint were assessed in the original study whereas they lack in the draft PEF Guide published in November 2011). The results will be used to discuss the degree of alignment of the PEF Guide with ISO 14040 and ISO 14044 as well as the practical implications of following the PEF Guide as compared to current LCA practice.
The PEF Guide aims at providing detailed technical guidance on how to conduct a PEF study identifying single requirement for each decision point with the objective to increase the consistency and the comparability of results whereas recognized methodological guidelines provide several alternatives, ranging from very general in the case of ISO 14040 and ISO 14044 to high specificity in the case of the French standard BPX30-323.
The Nespresso pilot test will be presented and shows an example of PEF Guide application and used to provide feedbacks about the PEF Guide.

TH 359
SETAC's influence on LCAs growth and direction
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The launch of Google labs' Books Ngram Viewer, which allows any user to graph the frequency of occurrence of words or phrases in Google's database of 500 billion words from digitized books, allowed for an investigation into the relationships of a number of LCA-related words over time, One combination, "SETAC"(Society of Environmental Toxicology and Chemistry) and "life cycle assessment", yielded a very interesting relationship for the years 1980-2008. The "SETAC"acronym first appeared in books in the 1980s. Its relative frequency of appearance grew steadily from 1990 through 2004, showing a tenfold increase. In 1990, SETAC sponsored an international workshop at which the term "life cycle assessment"was coined. Before then, a few practitioners in the U.S. and Europe used different terms such as "Resource and Environmental Profile Analysis"("REPA"). SETAC established the accepted name (and framework) for life cycle assessment. The occurrence of the phrase "life cycle assessment"in books grew very similarly to the occurrence of "SETAC"from 1990 through 2004. Was this a coincidence or were there activities within SETAC that contributed to this parallel growth? This paper will provide a perspective of the role of SETAC in both NA and Europe had on the development of LCA.

THPC1-1
Occurrence of pharmaceuticals in several wastewater effluents and evaluation of a non-conventional biodegradation treatment
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Among the vast array of contaminants of anthropogenic origin reaching our water supplies, pharmaceutically active compounds (PhACs) have one of the largest inputs into the environment. The main route of entrance into the aquatic environment is ingestion following excretion and disposal via wastewater, manufacturing and hospital effluents, land applications (e.g. biosolids and water reuse), concentrated animal feeding operations (CAFOs) and direct disposal/introduction into the environment. Many studies have reported on the limited degradability of pharmaceuticals under the conventional treatments applied in WWTPs, concluding that it is necessary to investigate on other possible ways of treatment to achieve high quality effluents. It would be desirable that selected treatment methods would be effective for as many different pharmaceuticals as possible. This goal could be achieved with fungal treatment; in particular ligninolytic fungi have a powerful enzymatic system which has demonstrated its ability to degrade a wide variety of xenobiotics.
In this context, the present work will show the results derived from an extensive characterization of 80 human and veterinary pharmaceuticals in several wastewaters where concentration of pharmaceuticals is expected to be significant such as hospital and urban wastewaters and reverse osmosis concentrate. Results indicated that pharmaceuticals are widespread pollutants in all these matrices. As expected, high levels were found in hospital wastewaters followed by those found in reverse osmosis concentrate and urban wastewaters (concentrations between high ng/L and high µg/L). Among all PhACs investigated, the iodinated X-ray contrast (ICM) agent iopromide was detected in all wastewaters, with especially high concentrations in hospital wastewaters (nearly 200 µg/L), whereas in reverse osmosis concentrate and in urban wastewaters levels where much lower (40 and 70 ng/L, respectively). The persistence of ICM through conventional wastewater treatment is well documented. For this reason, this substance was selected as target compound to study its degradation and to assess the potential of white-rot-fungi to degrade PhACs from real effluents.

THPC1 - Wastewater effluent discharges: chemical characterisation and understanding potential risks in receiving waters

THPC1-2
Possible removal of several contaminants from the wastewaters by a natural biofiltration process
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Many recent studies have revealed the presence of concentrations of hundred pharmaceuticals and illicit drugs in the high ng L-1 to low µg L-1 range both in surface and sewage waters. Since more of them are only partially removed by the sewage plants, they contaminate the receiving surface waters with potential implications for wildlife. Moreover, some sewage effluents are used for agricultural purposes and they can transport these pollutants to soil and cultivated plants also. The aim of this research is the evaluation of possible abatement of these chemicals in sewage waters by the use of a biological process. In detail, we built a pilot-plant (600 L of volume) in the biggest depuration plant (Depuration plant of Nosedo) of Milan (Italy) in which we put several thousand of specimens of the freshwater bivalve Dreissena polymorpha. Mussels were attached to Plexiglas panels that force the waste to follow a zigzag pathway. We exploited two natural behaviors of this mussel: the heavy filtration rate (average of 200 mL/mussel/h), which transfer the contaminants from waste to the bottom of tank by the production of faeces and pseudo-faeces, and the bioaccumulation capability of lipophilic chemicals in mussel soft tissues. Thus, by the cyclic elimination of faeces and contaminated mussels from tank, we may depurate the waste. We will measure the possible clearance of several environmental pollutants: pharmaceuticals, illicit drugs, some persistent organic pollutants (POPs), several heavy metals and pathogen bacteria. Our project plan foresee three different steps: 1) the evaluation of the capability of D. polymorpha specimens to attach themselves to panels and to live in wastewater 2) to test the possible decrease of pollutants in re-circulation conditions; 3) to check the contaminant abatement from the inlet to the outlet of pilot-plant. In the first phase, we collected from two Italian lakes about 100,000 mussels that were put in two "nursery"tanks filled with tap water in which the Plexiglas panels were horizontally positioned. We evaluated the subsequent attachment and the optimal conditions for the mussel sustentation (type of food, water flux, temperature, mussel density). Secondly, panels with the attached mussels were put vertically into the pilot-plant to carry out the second step, which is in execution at this moment.

THPC1-3
A modified clay material as cost-effective sorbent for waste printing developer decontamination
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The application of moderated fired clay with polymer addition to the purification and treatment of printing wastewater may potentially revolutionize water treatment processes. The efficiency of treatment technologies at removing specific substances or groups of substances, especially those identified as priorities under the WFD is confirmed with Annex II lists of the waste and pollutants covered by the register, which include greenhouse gases, acid rain pollutants, ozone-depleting substances, heavy metals and certain carcinogens such as dioxins. Nanotechnology exploits the novel phenomena and properties of matter at atomic and molecular levels. Because of their particle size they can exhibit an array of novel properties that can be used to develop new water treatment technologies and improve existing ones.

Clay derivative materials with high adsorption capacities are very attractive from an economical point of view. Some naturally occurring clay minerals may serve as cost-effective sorbents for the removal of Zn(II) ions. While their sorption capacity is usually less than those of synthetic sorbents, these materials could provide an inexpensive substitute for the treatment of printing wastewaters. To enhance the sorption capacity, the clays are modified with various polymer additions. However, due to the economic constraints, a development of cost effective and clean processes is desired. Adsorption processes has proved to be the most effective, especially for effluents with moderate and low heavy metal concentrations, as like as in waste printing developer.

The present work was undertaken to investigate the feasibility of the cost effectiveness adsorbents: pure fired clay, newly designed fired clay with the polymer addition (5 mass% PEG 600) and bentonite for Zn(II) ion removal from waste printing developer. The adsorption efficiency and distribution coefficient of used adsorbents were determined as a function of adsorbent amount. The results show that newly designed clay adsorbent with polymer addition has great potential (up to 93.5%) to remove Zn(II) ion from waste printing developer.
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THPC1-4
Remotion of POPs from industrial wastewater using UVC/H2O2 Photolysis
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In this study use of H2O2-assisted TiO2 photocatalytic degradation was applied to remove persistent organic pollutants from industrial wastewater. Experiments were carried out with treated and filtered (0.7 µm) urban wastewater, spiked with AZO Orange II (5mg/l) and 4-Clorophenol (0.36mg/l).
The experimental equipment employed in the assays consists in a tubular reactor of borosilicate glass, (600ml) operating in a recirculating circuit, irradiated with an UVC lamp (8W). The Hydrogen Peroxide (H2O2) in the process was 200mg/l. The catalyst (TiO2) was immobilized on the external surface of a cylinder of frosted glass (C=40mm). Experiments have been conducted using a total working volume of 1200ml and a recirculation flow rate of 600 (ml/min).
The efficiency of primary degradation was determined by monitoring the process, for AZO Orange II (absorbance 478 nm) and 4-Clorophenol compounds. Toxicity assays were also carried out with the aim to evaluate the effects by products. An economic study of this process was included in the global analysis.

THPC1-5
Impacts of advanced wastewater treatment on metal speciation and bioavailability
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Increasing pressure to remove priority chemicals from wastewater treatment effluents is leading to pressure to utilise or consider advanced tertiary treatment options such as UV radiation, filtration, GAC, and ozonation as part of the wastewater treatment process. The use of these adsorptive or destructive methods may, however, increase, decrease, or leave largely unchanged the concentrations of ligands in effluents that are important for complexing metal ions and which, consequently, reduce the toxicity potential of metals such as copper and zinc. Since there is not much data on the metal complexing characteristics of effluents before and after tertiary treatment, there is uncertainty in the extent by which tertiary treatment options might impact metal bioavailability. To investigate possible impacts, a chelex column ion exchange method was used to assess the effects of GAC, ozonation, and UV radiation in combination with H2O2 on the ligands in sewage effluent that are responsible for complexing copper and zinc. Preliminary results indicate that tertiary treatment options tend to increase the concentrations of metal complexing ligands relative to the concentration of dissolved organic carbon present. This effect was also of greater significance for copper than for zinc. The ozone treated effluents displayed lower UV absorbances (350nm) in relation to other secondary and tertiary treated effluents, indicating ozonation to have some impact on the concentrations of aromatic substances in the effluents, which is consistent with the elimination of humics; however, this did not impact complexation capacity. These findings indicate that advanced tertiary treatments are unlikely to increase the bioavailability of metals in sewage effluents.

THPC1-6
The influence of terminal electron acceptor on the removal of pharmaceuticals in Anaerobic digester sludge
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Within each stage of the wastewater process pH and redox conditions fluctuate, and the characteristics of each sludge vary. These changes result in differences in the microbial population present, and variations in how strongly micropollutants adsorb to each sludge. The total removal of micropollutants, the rate of removal, and the mechanisms responsible for removal will therefore vary depending on the redox conditions present.
Anaerobic digestion is used for the treatment of 2/3 of municipal biosolids within the UK, and is a growing technology for the treatment of industrial and food waste due to the generation of renewable energy in the form of biogas. Anaerobic digestion is a multi-step microbiological degradation process, and can be performed in a single reactor or in multiple reactors where the hydrolytic fermentation and methanogenic phases are separate. Hydrolysis, acidogenesis, acetogenesis and methanogenesis each occur under different redox conditions, and each is associated with a different terminal electron acceptor (nitrate, suphate, carbonate and methane respectively).
Separating hydrolysis from methanogenesis has been shown to be more efficient for the generation of biogas, however, biodegradability tests performed to determine whether a test substance will degrade under anaerobic conditions are performed under methanogenic conditions only. A test system whereby the redox potential is controlled through amendment of terminal electron acceptors was validated through the measurement of redox potential (Eh), the reduction of carbonate, nitrate and sulphate, and the removal of dissolved organic carbon. When it was demonstrated that these conditions could be effectively controlled this test system was applied to the removal of test substances under amended conditions.
Conditions were amended in a batch test and in a serum bottle experiment to measure the total removal and mineralisation of two prioritised test substances that have been identified within the biosolids. The results of these experiments will be discussed and compared with data obtained from biodegradability experiments performed under unameded conditions following guideline OECD 311.

EP06P - Perfluorinated compounds: From emission sources to the place of impact

TU 001
Particle size distribution of airborne perfluorinated compounds
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In recent years, perfluorinated compounds (PFCs), particularly perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) have been described as compounds of increasing environmental concern and numerous studies were conducted to investigate the environmental distribution and fate of these compounds Besides their transport with ocean currents, atmospheric transport was identified as important (long-range) transport pathway. As the particle size is an important parameter characterizing the fate of atmospherically transported compounds, knowledge about the particle size distribution of particle-bound PFCs is of particular interest. Therefore the objective of this study was to investigate the particle size distribution of PFCs.
Particle phase samples were taken in parallel using a cascade impactor with cut-off diameters of 11.4 µm (A4, B4), 3.81 µm (A3, B3), 1.38 µm (A2, B2), 0.46 µm (A1, B1), and 0.14 µm (back-up filter, A0, B0). Each particle size fraction of each sampler was extracted separately using ultrasonication and methanol. Samples were measured by HPLC-ESI-MS/MS.
PFC concentrations in particle phase samples (sum of all fractions) were between 0.5 and 2.5 pg m^{-3}. Of 25 analysed PFCs, 18 could be quantified. These were C4, C6-C8 PFSA, C4-C14 PFCA, PFOSA, MeFOSE and EtFOSE. PFOS and PFOA were observed in highest concentrations (0.17-3.5 pg m^{-3}, 0.09-4.8 pg m^{-3}, respectively), followed by PFBA and PFNA.
The observed particle size distribution of PFCs in parallel samplers were quite similar indicating a good precision of the entire method. However particle size distribution between perdluoroalkyl sulfonates and perfluoroalkyl carboxylates differed. Whereas carboxylates were primarily detected in the finest particle fraction (<0.14 µm) sulfonates were mainly observed in mid-size particle fractions (cut off 3.81; 1.38) of the investigated particle size spectrum.

TU 002
Binding and sequestration of perfluorinated surfactants in soil
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Perfluorinated surfactants (PFCs) produced since the 1950s draw great attention due to their wide application and increasing occurrence in the environment. Sorption coefficients (Kd) and organic carbon normalized soil adsorption coefficients (Koc) are essential for determining soil adsorption behavior and assessing the risk of transfers of these chemicals from contaminated soils to plants and groundwater. We therefore analysed the international literature for concentrations of pefluorinated octanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in different environmental media (surface water, sewage treatment plants, sediment, and soil) in order to assess the distribution of PFOA and PFOS between these environmental compartments and compared the results with published experimental sorption coefficients. Experimental log Koc values for PFOS for different soils and sediments available in public literature are remarkably similar with a coefficient of variation of only 18%, which should limit the uncertainty of concentrations in drainage water and groundwater calculated from soil concentrations (average log Koc = 2.9). For PFOA however, published sorption coefficients are sparse and the published information regarding the analogy between sorption of carboxylated and fluorinated PFCs is controversial (average log Koc = 2.6). Our review shows that current pattern of environmental PFOA and PFOS concentrations are strongly influenced by local pollution sources. Nevertheless, we calculated "global"Kd/ Koc distribution coefficients based on median concentrations in sediments/surface waters or sewage sludge/sewage. The "global average"Kd calculating from the median concentrations of PFOA in WWTP effluent and sewage sludge equals 1958 l kg-1 (log Kd =3.3). Assuming an average dry matter organic carbon content of 31% for sewage sludge would give a global average log Koc of 3.8. Dividing the median sediment concentration of 0.6 ng g-1 by the median surface water concentration results in a "global average"Kd value of 275 (log Kd = 2.5). The "global average"log Koc assuming an average sediment Corg concentration of 1.7% equals 4.2. Furthermore, the estimated "global"log Koc were similar to experimental log Koc values found in the literature. The moderate affinity of PFOA and PFOS to soils, sediments and sewage sludge indicates that drinking water must be considered a potential pathway of exposure of animals and humans to these compounds.

TU 003

Investigating the sorption of Perfluorinated Compounds in different types of sludge

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Perfluorinated compounds (PFCs) present significant research interest in recent years due to the global distribution, persistence, bioaccumulation and potential toxicity of these compounds. Despite the fact that PFCs are commonly detected in municipal wastewater treatment plants (WWTPs), so far there are few data for their sorption potential to sludge. The aim of this research was to investigate the sorption potential of different PFCs to three different types of sludge (primary sludge, secondary sludge and digested sludge). For this reason, sorption experiments were performed with eight PFCs belonging to two different classes; perfluorocarboxylic acids (PFCAs) that contain a carboxylic group in their molecule and perfluoroalkyl sulfonates (PFASs) that contain a sulfonate group in their molecule.

At first, sorption experiments were performed to investigate the equilibrium time for each target compound and the role of sludge inactivation on sorption potential of PFCs. For this reason, samples were taken at different time intervals and PFCs were detected in dissolved and particulate phase. PFCs were extracted by solid-phase extraction (dissolved phase) or sonication (particulate phase) and determined using High Performance Liquid Chromatography - Ion Trap Mass Spectrometry (HPLC-IT-MS). Afterwards, sorption experiments were performed for six different spiking levels of PFCs, ranging between some ng L-1 to few μg L-1 and solid - water distribution coefficient (Kd) values were calculated. Finally, the effect of solution pH on PFCs sorption was investigated in experiments performed at pH values commonly found in WWTPs (6, 7 and 8).

According to the results, Kd values of PFCs were differentiated significantly according to the type of sludge. Moreover, it was shown that both the length of the perfluorocarbon tail and the functionality of the head group can influence the sorption of these compounds to sludge materials.

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TU 004

Presence and behaviour of perfluorinated compounds in sewage sludge

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Perfluorinated compounds (PFCs) are used in industrial and domestic appliances and have emerged as significant global environmental pollutants with persistent, bioaccumulative and toxic properties. After use or disposal of PFC-containing products, these compounds enter Waste Water Treatment Plants (WWTP) and accumulate in sludge because their intrinsic physicochemical properties make them resistant to final mineralization. Among other options, the use of sewage sludge as agricultural fertiliser is common practice all over Europe. The aim of this study was to determine the presence of 5 perfluorinated compounds (PFCs), namely perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHxS), perfluorobutanesulfonate (PFBS), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) in sludge and evaluate their transfer to agricultural soils. PFCs were analyzed in sludge from 15 WWTP from Spain and Germany. These WWTP receive both urban and industrial wastes. PFCs were detected at 0.28 and 5.15 ng/g dw for Spanish sludge and from 14.2 to 56.0 ng/g dw for German Sludge, with differing concentration patterns of the individual compounds. In addition, the degradability of PFCs within the sludge treatments (primary sludge, anaerobic digested and centrifuged sludge) was evaluated and we found that anaerobic digestion and centrifuging did not have an effect on the PFC elimination. Subsequently, the amount of PFCs accumulated per ton of sludge generated was determined for each WWTP. According to the use of sludge in each country, the calculated contribution of PFCs to agricultural soils was estimated between 0.06 and 12.88 g/ha month. Finally, the concentration of PFCs were determined in agricultural soils and sludge amended soils as a way to determine the real impact of PFCs in soils. Although there are no legislated limits for PFCs in sludge (Council Directive 86/278/EEC and Royal Decree 1310/1990, regulating the use of sewage sludge in agriculture), its use in agriculture may lead to diffuse pollution of soils and groundwaters. In Spain, 95% of the sewage sludge is used as fertilizer in agriculture while in Germany, most of the sludge is incinerated or used for landfilling.

TU 005

Occurrence and evaluation of the fate of Perfluorinated Compounds in wastewater treatment plant of Athens (Greece)

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Perfluorinated compounds (PFCs) have been classified as emerging contaminants and they are commonly detected in municipal wastewater treatment plants (WWTPs). In this study, contamination profiles of eighteen target perfluoro analytes (C5 to C14 carboxylic acids, C4, C6-C8 and C10 sulfonic acids and 3 sulfonamides) were detected in wastewater (influents and secondary effluents) and sludge samples, originating from WWTP of Athens (Greece).

Two seasonal sampling campaigns were performed and samples were taken in seven consecutive days in each sampling campaign. The analytes were extracted by solid-phase extraction (dissolved phase) or sonication (solid phase). Qualitative and quantitative analyses were performed by LC-MS/MS. Recovery values generally ranged between 80% and 115%, while lower recoveries were obtained for longer perfluorocarboxylic acids (PFCAs) and perfluoroalkyl sulfonates (PFASs) in sludge samples. The limit of quantification of the target compounds varied from 0.29 to 3.0 ng L-1 and from 0.15 to 1.5 ng g-1 for liquid and solid samples, respectively. The seasonal and daily variations of target compounds' concentrations were investigated and the results were compared to those reported in the literature for other WWTPs worldwide. Daily mass flows normalized to inhabitants were also calculated and distribution of target compounds between dissolved and particulate phase was determined for wastewater samples. A mass balance was also performed to investigate the fate of PFCs during wastewater treatment plants and to estimate the role of degradation and sorption on their removal.

According to the results, perfluorooctanoic acid (PFOA) and perfluoroctane sulfonate (PFOS) were detected in all samples and they were the major contaminants in wastewater and sludge. Mean concentrations in influents, effluents and dewatered sludge ranged up to 90.4 ng L-1 (PFOA), 103.5 ng L-1 (PFUdA) and 377.9 μg Kg-1 (perfluoroundecanoic acid, PFUdA), respectively. Excepting perfluorooctane sulfonamide (PFOSA), perfluoropentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA) and perfluorodecanoic acid PFDA that were partially removed during wastewater treatment, concentrations of other PFCs in effluents were similar or higher comparing to influent wastewater. The observed increase in concentrations of some PFCs in effluent wastewater, suggests a possible formation of these compounds within the wastewater stream due to biodegradation of precursor compounds.

TU 006

Biodegradation of proposed PFOA & PFOS precursors in aerobic batch assays

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Perfluorinated compounds (PFC) are a group of emerging environmental pollutants even though no natural sources of PFC have been proposed. Nevertheless they can be detected in the environment throughout the world. Due to their unique water-, fat- and oil-repellent as well as stain-resistant properties, they are widely used in various industries and commercial products. Some of them are classified as persistent, bioaccumulative and toxic compounds. Hence they display a threat of nature. Potential sources of PFC can be consumer and industrial products as well as manufacturing processes.

In some sewage treatment plants worldwide, an increasing mass flow of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) was observed with higher concentrations in the effluents compared to measurements in the influents. This leads to the assumption that there have to be precursors which are biodegraded during the sewage treatment. Thus the aim of this work was the detection of precursors for PFOA and PFOS by studying their aerobic biodegradability.

Amongst others perfluorooctanesulfonamide (PFOSA)[1] , 8:2 telomeric acid (8:2 FTCA), 8:2 telomeric alcohol (8:2 FTOH)[2,3] , and a technical product containing polyfluoroalkyl phosphates were chosen to study their fate in aerobic batch assays. The test period was 14 days each at a water temperature of 12.7°C. The activated sludge samples were obtained from a municipal sewage treatment plant and the test medium was chosen following the EN ISO 9888 guideline, June 1999, with a 10-times higher phosphate buffer concentration. The water and sludge samples were analyzed using LC-MS/MS and GC-MS.

All in all the above mentioned substances degraded to PFOA or PFOS in ranges from 1 to 8 per cent during 14 days. The lowest metabolic rate was monitored for PFOSA. In addition the technical product degraded to perfluorohexanoic acid (PFHxA) as well. The absence of degradation products in the abiotic controls show that the metabolism has to be attributed to microbial degradation only.

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TU 007

Identification of the sources of polyfluoralkyl substances (PFASs) in the Italian surface waters

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A survey of the distribution of perfluorinated carboxylates (from C5 to C10) and perfluorinated sulphonates (C4 and C8) in the main Italian river basins have been carried out in 2011 in order to get a reliable picture of the polyfluoralkyl substances (PFAS) contamination and possibly to identify the main sources for the aquatic environment. Monitoring campaigns on river Po (north of Italy), the major Italian river which flows in the Adriatic Sea, its tributaries, river Adige, river Tevere, river Arno and river Brenta basin have been carried out in different hydrological conditions. A survey has been performed also in transitional coastal areas such as river Po Delta and Lagoon of Venice. Chemical plant discharges and drinking waters present in the same basins were also sampled.

Analyses of PFAS were achieved by using an on-line SPE-HPLC-MS-MS system. Recovery for PFASs for all analytes was above 70% at μg/L levels. Limits of detection (LOD) ranged from 0.2 to 2.5 ng/l.

The monitoring campaigns allowed to identify hot spots in the main Italian basins.
Unlike rivers Tevere and Adige which are not significantly impacted, concentrations determined at the basin closure of the river Po are comparable to those measured in the PFAS heavily impacted areas of northern Europe: PFOS concentrations are low (ranging from < LOD to 2.5 ng/L) while PFOA is the main compound (about 25 ng/L). Fluoropolymer factory, present in the Tanaro/Bormida watershed, was confirmed as the main source of the latter molecule. Plant for the production of fluorochemicals , used as intermediates in the polymer synthesis, is a significant source of PFOA and PFBS in the river Brenta which discharges in the Adriatic sea.
Two important textile industrial districts in Italy (counties of Vicenza and Prato) have been also identified as a significant source of PFPeA and PFHxA which are discharged in the Brenta basin and in river Arno. These shorter chain perfluorocarboxylics acids can be considered as tracers for this kind of industrial pressure. The same compounds were also measured in the river Adda, an important tributary of the river Po, but the source shall be still to be identified.
The diffusion of these substances in the drinking waters in the same areas has been also studied.

TU 008
PFAA sources to groundwater and drinking water: identification and origin
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All humans are exposed to perfluoroalkyl acids, as has become evident from blood analysis. The exposure to PFAA occurs primarily via the dietary intake and more specifically via drinking water. It was estimated that when assuming a tap water concentration of PFOA of 9 ng/L the intake via water would amount to 55 % of the total daily Dutch PFOA intake.
Knowledge about sources of PFAA to drinking water is currently scarce and focuses on surface water based drinking water. Waste water treatment plants have been identified as major sources for PFAA to surface waters and ultimately to the drinking water produced from it. However, groundwater is in many countries the major source for drinking water production (in NL 65%). The extent of contamination of groundwater by PFAA and the contamination pathways to groundwater bodies remain largely uncertain.
In the work presented, we identified different direct sources of PFAA to groundwater within the catchment area of a public supply well field (PSWF) in the Netherlands. It was found that landfill leachate, and urban/military base affected rainwater contaminated the groundwater within the catchment area of the PWSF. Rainwater not affected by the direct sources also infiltrated in this area and provided a background contamination to the groundwater.
Maximum concentrations encountered in the landfill leachate plume were 1758 ng/L perfluorooctanoic acid (PFOA) and 1165 ng/L perfluorobutanoic acid (PFBA). Maximum concentrations measured in the groundwater halfway the contaminant sources and the PWSF (15 years travel distance) were 29 and 161 ng/L for PFOA and PFBA respectively. Concentrations in the groundwater wells at a travel distance of 25 years were much lower: 0.96 and 3.5 ng/L for PFOA and PFBA respectively. The chemical signature of the groundwater showed that infiltrated rainwater, the landfill leachate and the infiltrated urban/military water contaminated the abstracted water. Based on the hydrological modeling it was shown that 1% of the pumped groundwater was originating from the landfill and military area and 99% from background contamination. This is seen in the relative abundance profile of the PFAA in the pumped groundwater.

TU 009
Seasonal distribution of perfluorinated compunds (PFCs) in surface water from Elbe River and North Sea, Germany
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Since the ubiquitous occurrence and potential toxicity to human beings, the perfluorinated compounds(PFCs) have attracted more concerning in the recent years. In 2010, one of the PFCs, perfluorooctane sulfonate(PFOS) was added to Annex B of Stockholm Convention on Persisitent Organic Pollutants(POPs). Recently, more substitutes, i.e. perfluorobutane sulfonate (PFBS) and perfluorohexanoic acid (PFHxA), were widely used and the elevated levels have been found all over the worlds. In this study, four cruises in Elbe River and three cruises in North Sea were conducted throughout the whole year of 2011 to investigate the seasonal distribution of perfluorinated compounds in surface water. Perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFSAs) were determined in both particle and dissolved phases in more than 100 samples. The profile patterns suggested the industrial and urban discharge which were the major source of PFCs in river and coast water.

TU 010
Occurrence of 14 perfluorinated compounds and other priority and emerging organic compounds in fishes from the Rhone River (France)
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The Daughter Directive ['EQS' Directive, 2008/105/CE; EC, 2008] details the application of the Water Framework Directive for the monitoring of priority substances and other pollutants in surface waters; in particular, it requires the implementation of temporal and spatial trend monitoring programs. The use of integrative matrices (biota and sediments) is strongly recommended to achieve such objective, especially for compounds having a log Kow> 3.
The main goal of this study was to obtain data on the occurrence and levels of 34 priority and emerging organic compounds in freshwater fish sampled in the Rhone River. The analyses included 14 perfluorinated compounds (PFC),and also 5 alkylphenols (AP), bisphenol A (BPA), 3 hexabromocyclododecanes (HBCD), 9 polybromodiphenylethers (PBDE) and 2 compounds, hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) for which Environmental Quality Standard (EQS) have been set for biota (10 and 55 µg/kg fresh weight -fw- respectively) [E.C., 2008].
Fishes were captured from August 2008 to January 2009 at 3 sites located upstream and downstream of the Lyon metropolitan (France). The four freshwater fish species studied, namely the barbel (Barbus barbus), the common bream (Abramis brama), the white bream (Blicca bjoerkna) and the chub (Squalius cephalus), were chosen because they may reach large size and are long-lived species, but they have different diets and exploit different habitats. Chemical analyses were conducted on pooled fish samples and also on some individual fish samples in order to check the homogeneity of pooled samples. A total of 49 fish samples were analysed (pooled or individual fish samples). Results on the quantification frequencies, mean, median, minimum and maximum concentrations measured for the 14 AP and the other selected compounds in the 49 fish samples will be discussed according to what has been already found in the literature. Comparison on contamination level according to the fish species and also to the sampling site will be presented and discussed.
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TU 011
Perfluorinated compounds (PFCs) in peregrine falcon eggs from southwestern Germany - levels, patterns and temporal concentration variations
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As a top predator, the peregrine falcon (Falco peregrinus) is capable of accumulating a wide range of persistent pollutants and therefore a valuable object for observation and chemical analysis. The species attracted not only scientific but also public interest, at the latest since it was at the brink of extinction in Germany and elsewhere through excessive burdens of organochlorine pesticides such as DDT. A network of volunteers in the German state of Baden-Württemberg offered the possibility to collect unfertilized or unhatched peregrine falcon eggs as samples for monitoring environmental contaminants. In the past years, perfluorinated compounds (PFCs), especially PFOS raised scientific and public concern as their POP-like properties became apparent. Therefore, the analysis of PFCs in peregrine falcons' eggs was integrated into the existing monitoring programme.
Samples were collected in Baden-Württemberg from 2008 to 2011. About half of the number of the 20-30 egg samples collected per year were analysed for 11 PFCs (4 sulfonates, 6 carboxylates and the perfluorooctane sulfonamide). The analysis was performed using cold sample extraction and ion exchange-SPE clean-up followed by LC-MS/MS detection at LOQs of about 0.5 ng/g dry weight.
For this poster we aim for presenting data in order to form a baseline for the PFC levels in the eggs samples for the years from 2008 to 2011. This will offer the first possibility to establish the temporal concentration variations for PFC in peregrine falcons from Baden-Württemberg.
For 2009 and 2010, the substance spectrum was clearly dominated by PFOS at average concentrations of about 150-260 ng/g dry weight. Longer chain carboxylates were observed at concentrations of about 1-30 ng/g dry weight, whereas the PFHxA, PFHpA and PFOSA were not detected. The dry matter content of the eggs was at around 20%. PFOS concentration in the highest contaminated eggs approaches the toxicity threshold (LOAEL 5000 ng/g fresh weight; NOAEL 500 ng/g fresh weight) for PFOS established for chicken eggs. Thus Peregrine falcon eggs may be compromised by PFOS if their sensitivity is similar to the chicken. These findings generally confirm results presented earlier (e.g. Holmström et.al., 2010, Sweden).

TU 012
PFASs in feathers of white tailed eagles (haliaeetus albicilla) from Greenland and Norway; useful for non-destructive monitoring?
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The white-tailed eagle (Haliaeetus albicilla), also referred to as white-tailed sea eagle (WTSE), is a large predatory bird from the Northern part of Eurasia. It is a top predator from the aquatic ecosystem and feeds mainly on marine fish, waterfowl and carrion.1 Because of its high trophic position, the WTSE is a very interesting biomonitoring species for studying accumulation of persistent organic pollutants (POPs).
In this study, our aim was to increase the knowledge on the analysis of perfluorinated aliphatic substances (PFAS) in biological tissues and specifically develop a reliable method to measure PFAS in feathers of this raptor species. Feathers have already been used successfully for monitoring of heavy metals, and recently also for POPs, but a reliable method for PFAS was not available and needed to be established3,4. The results gained from this research enabled us to monitor endangered raptors without harming the birds and their offspring and to better understand the fate of PFAS in birds.
From Greenland body feathers (n=11) and primary wing feathers (2th, 5th and 8th primary; n=46) and preen oil (n=7) was collected when available in a sufficient amount for analysis. Tissue samples were taken from the Greenland carcasses to analyze for PFAS and POPs5. In addition, tail feathers were sampled at active nest sites of WTSE situated in Northern Norway (n=18) in 2009.
For the first time, PFAS were detected in feathers and preen oil of white tailed sea eagles from Greenland and Norway. In the wing feathers from Greenland WTSE, PFOS and PFOSA were detected. In some cases, PFNA, PFUnA and PFTrA were detected in minor concentrations as well. The average concentrations of the primary wing feathers #2, 5 and 8 were similar. In addition, the body feathers showed similar PFOS levels, but lower PFOSA levels.

TU 013
Perfluorinated chemicals in Belgian barn owls (Tyto alba): comparison of levels in feathers and tissues
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Feathers have proven very useful regarding non-destructive biomonitoring of contamination with heavy metals and organic pollutants. For this study, we have investigated perfluorinated chemicals (PFCs) in feathers and tissues of barn owls (Tyto alba), collected in the province of Antwerp (Belgium). A major PFC plant (3M) is located in the close vicinity of the city of Antwerp and levels of PFCs in biota from this area have been found to be very high in previous studies. We analysed levels of PFCs in tail feathers and tissues (liver, muscle, preen gland, adipose tissue) obtained from barn owl road-kill victims (n=15). We used methods for left-censored data to cope with levels below the limit of detection. We aimed to study the main sources for PFCs in feathers as levels can both originate from internal sources (via the blood) as from external contamination (via e.g. air, dust).
PFOS was detected in all tissues (median concentration: 135 ng/g in muscle, 305 ng/g in liver, 431 ng/g in preen oil, 203 ng/g in adipose tissue), also in feathers (median 14.7 ng/g; < 2.2 - 56.6). PFOS levels in feathers and liver tissue were highly correlated ($r=0.79$, $p<0.001$), but this was not the case for PFOS levels between feathers and muscle ($r= 0.15$, $p=0.47$) or between muscle and liver ($r=0.46$, $p=0.08$). This may be due to the chemical properties of PFCs which are mainly bound to proteins in the blood, thus reducing the suitability of muscle tissue. PFOA was measured at high levels in the feathers (<14.1 - 670 ng/g), but not in the tissues (more than 50% < LOD). Furthermore, PFOA levels in feathers and liver were not significantly correlated ($r=-0.17$, $p=0.57$). These results suggest that PFOA may be present on the external surface of the feathers and was not washed off by using distilled water and hexane. External contamination with PFOA is most probably originating from the air due to the close vicinity of potential point sources. PFHxS could only be quantified in liver and preen oil. Sporadically, other PFC compounds were detected as well, mostly in liver.
Overall, these results indicate that PFOS levels in feathers and liver are highly correlated, while PFOA levels in feathers are probably originating from external contamination. Therefore, the feasibility of feathers for PFCs monitoring should be investigated more in depth in the future.

TU 014
Monitoring of perfluorinated compounds
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Because of their persistence and wide ranging application perfluorinated compounds (PFC) are found as ubiquitous contaminants in aquatic and terrestrial organisms as well as in food products. HPLC-MS/MS analysis of animal and non-animal foodstuffs and wild game was performed within the framework of preventative consumer protection.

Fish from regional waters:
Sixteen fish from the Rhine river including eel, perch, roach, tench and catfish were examined for the presence of PFC. Concentrations between 5.2 µg/kg and 143 µg/kg were detected in muscle tissue as the sum of PFC detected (∑ PFBS, PFPeA, PFHxA, PFHxS, PFHpA, PFOA, PFOS, PFNA, PFDA, PFDS). The highest concentration was measured in perch muscle tissue.

Roe deer:
Use of PFOS has been declining since the year 2000. In order to observe the ensuing temporal changes in PFC concentrations in animal matrices a retrospective study of liver tissue from roe deer was undertaken. A total of 110 samples taken between 1998 and 2010 from the German Environmental Specimen Bank were examined. A decline in PFOS contamination since the beginning of this millennium is evident. Average PFOS concentration was 9.2 µg/kg in the year 2000 and 1.9 µg/kg in 2010.

Wild boar:
PFOS and PFOA concentrations were measured in 506 muscle tissue samples and 529 liver samples from wild boar. The arithmetic mean of PFOS concentrations detected in muscle tissue was 1.38 µg/kg whereas the mean PFOA concentration was below the LOQ. In liver tissue the mean PFOS concentration was 4.02 µg/kg (Maximum value 45 µg/kg) (1).

Foodstuffs:
Measurements of 82 samples of French fries showed PFC concentrations above the LOQ in 3 samples. No concentrations above the LOQ were detected in 30 samples of ice cream, 14 samples of whole milk, 19 samples of carrots or 16 samples of grains. A total of 84 samples of ocean fish, farmed fish, seafood, fish in packaged salads and canned tuna were tested for the presence of PFC. No PFC were detected in 82 of the samples. Two carp from fish farms had 2 and 14 µg/kg, respectively in muscle tissue.

(1) T. Stahl, S. Falk, K. Failing, J. Berger, S. Georgii, H. Brunn, Article title: PFOA and PFOS in Liver and Muscle Tissue from Wild Boar in Hesse, Germany, Arch Environ Contam Toxicol, DOI: 10.1007/s00244-011-9726-3

TU 015
Development of a pharmacokinetic model (PBPK) for the assessment of infant exposure to PFOS and PFOA for health risk assessment
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Perfluorinated compounds (PFCs) are very stable compounds with a lot of industrial application, but also very persistent on the biota and the environment. Among them, PFOS (perfluorooctane sulfonate) and PFOA (perfluorooctanoic acid), have a long persistence in the human body (4-6 years). This persistence creates an important concern among the scientific community and the health authorities. In fact, PFOS was listed under the Stockholm Convention in May 2009 as a persistent organic pollutant (POP), while PFOA is a serious candidate to enter that list. The main target organs of accumulation are blood and breast milk. Thus, PFCs can be a risk for adult population and infant. In order to assess the tissue concentration, PBPK models are able to assess the concentration along the time in the main target organs of concern. PBPK models are mathematical representations of the human body where the organs are considered as compartments, and they can be resolved computationally as a set of equations.
The PBPK model presented here is a multi-compartmental model for PFOS and PFOA for adult and childhood exposure during the breastfeeding period. Aside from the blood and breast milk, another tissue such as brain and kidney have been considered. PFCs are well absorbed, non metabolized and eliminated by urine and breast milk. The main sources of exposure for adult population are food and water intake. The data for food and water ingestion have been obtained from a large monitoring study in Catalonia, Spain.
For toxicity, the tissue residue approach (TRA) was used. The TRA is the use of tissue concentrations as the dose metric for characterizing toxicant potency. The PBPK model here presented was validated by comparing theoretical values with experimental data of PFCs chemicals in blood serum and breast milk collected in Catalonia, Spain. Finally, uncertainty and sensibility analyses have been performed. A normalized local sensitivity analysis was performed on the model to examine the influence of each model parameter on the model output. Sensitivity coefficients were calculated for the predicted plasma area under the curve (AUC; total concentration) with the original parameters and for those resulting from a low change in each parameter value.

TU 016
Acute toxicity of Perfluorinated compounds to two kinds of cladocerans
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Due to their unique properties, poly- and perfluorinated compounds (PFCs) have been manufactured and used during the past 50 years in a variety of industrial and commercial products, such as household surface finishes, food packaging, water- and stain-resistant materials, and fire-fighting foams. PFCs were released to the environment during production, usage and disposition. PFCs tend to persist in surface waters. Therefore their toxicity to aquatic organisms is of particular concern. Serving as a food source for amphibians, fish and other aquatic organisms, cladocerans are one of the key trophic elements of aquatic ecosystems. In addition to their ecological significance, cladocerans have the advantages of being useful as test organisms due to their short life cycle, their ease of laboratory culturing, their limited space and water volume requirements, and their sensitivity to chemicals. Cladoceran species are therefore widely used in aquatic toxicology. Among freshwater cladocerans, Daphnia magna is probably the most commonly used test organism in ecotoxicological studies. Some toxicity tests have been performed on cladocerans for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) or their salts.

Because of their global distribution, environmental persistence and potential risk to human beings and aquatic organisms, PFCs are of particular concern for research and regulatory communities. However, insufficient toxicity data are available to accurately assess their possible environmental hazard. Therefore, the acute toxicity of seven poly- and perfluorinated carboxylic acids and alcohols on two cladocerans, Daphnia magna and Chydorus sphaericus, was evaluated in the present study. The adverse effects of these PFCs on these two cladocerans decreased with increasing fluorinated carbon chain length (nC) and quantitative structure-activity relationships were developed to quantify this observation. As the EC50 values obtained are far above concentrations typically found in surface water, acute harmful effects of these chemicals to D. magna and C. sphaericus are not expected in the real environment.

TU 017
Perfluorooctanoic acid toxicity in zebrafish (Danio rerio)
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Perfluorooctanoic acid (PFOA) is a common surfactant with wide use due to its unique nature, and has been detected as a contaminant in soil, air, water and biota. Previous studies have shown that PFOA has detrimental effects on different life stages in zebrafish (Danio rerio).

We have evaluated the effects of PFOA toxicity covering different life stages of zebrafish through three tests that were based on OECD guidelines. In the Fish Embryo toxicity (FET) test, fertilized embryos were used to calculate effective concentrations (LOEC and NOEC) based on early life stage lethal and sub-lethal endpoints. Based on the FET test, a Fish Sexual Development Test (FSDT) was conducted and effects on vitellogenin (VTG), body and liver somatic indices and gonad histopathology including sex ratios were evaluated. A reproduction test (Fish Screening Assay: FSA) was also performed where effects on reproductive parameters like spawning, fecundity and fertilization rate were evaluated, as well as effects on histopathology, liver enzyme activity VTG-levels and key genes expression levels in the adult fish.

TU 018
Structure-activity relationship of four perfluorinated compounds based on early developmental effects in the zebrafish
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Perfluorinated compounds (PFCs) are a group of anthropogenic chemicals containing diverse functional groups and chain lengths. Their chemical structure gives them unique properties which resulted in massive production and the use of these compounds in several familiar products since the 1950s. However, these unique properties also cause them to be persistent and bioaccumulative explaining their presence in wildlife, humans and the environment worldwide. Despite the global occurrence of PFCs, the toxicological information on these chemicals is still incomplete and insufficient to assess their environmental impact and structure-activity relationship. In the present study, the developmental effects of PFOS (perfluorooctane sulfonate, C8), PFOA (perfluorooctanoic acid, C8), PFBS (perfluorobutane sulfonate, C4) and PFBA (perfluorobutanoic acid, C4) were evaluated in zebrafish embryos (Danio rerio) during 120 hours post fertilization (hpf). The different chain lengths and functional groups of the selected chemicals made it possible to determine the structure-activity relationship of these compounds. PFCs with longer chain lengths (C8) tend to be more toxic than PFCs with shorter chain lengths (C4). Comparison based on the functional groups of compounds with the same chain length indicates that PFCs with a sulfonate group have a larger toxic potential than the ones with a carboxyl group. This resulted in the following order of toxicity: PFOS>PFOA>PFBS>PFBA. Furthermore, exposure to the different PFCs resulted in some general effects, such as deformations of the tail and an uninflated swim bladder, as well as in more specific effects depending on the structure of the tested chemical. Oedemas and effects on length could only be detected in 8-carbon PFCs while malformations of the head were a more specific action of the sulfonated PFCs. Only PFOA caused significant effects on hatching rate and success. The effects of PFOS, PFOA and PFBS on the heart rate at 48 hpf could be the result of effects on intrinsic processes that control the heart rate, while altered heart rates at 72 hpf can be attributed to the shorter body lengths. In conclusion, PFCs with short chain lengths should be primarily considered throughout the development of new perfluorinated alternatives.

TU 019
Embryotoxic effects of perfluorooctane sulfonate compounds in sea urchin Paracentrotus lividus
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Our environment has been affected by increasing concentration of organic and inorganic pollutants from anthropogenic sources. Perfluorinated compounds are important source of pollution and they are major risks for the aquatic ecosystems. Perfluorooctane sulfonate (PFOS) and Perfluorooctane sulfonyl fluoride (POSF) are both industrially synthesised perfluorinated chemicals and they produced in substantial amounts. They are found widely in surface water and aquatic sediments. In this study, the comparative embryotoxicity of increasing concentrations of PFOS (five concentrations ranging from 0.5 to 10 mg/L), and POSF (five concentrations ranging from 0.5 to 10 mg/L) were examined on embryos of the sea urchin *Paracentrotus lividus*. Results were evaluated by observing: larval malformations, developmental arrest and embryonic/larval mortality. The results indicated that, low concentrations of these chemicals generally caused malformations in the skeletal system. It was observed that high concentrations (10 mg-PFOS/L, 10mg-POSF/L) inhibited the growth of embryos in the early life stages by preventing mitosis. As a result, POSF was more toxic than PFOS (EC_{50}: 1.795 mg/L) on sea urchin embryo as the EC_{50} is 1.074 mg/l and also both of the chemicals were harmful on the embryonic development of sea urchin in the sense of whole embryo and cellular level. In conclusion PFOS and POSF present a major risk to the normal development of *P. lividus* in the environment, so that it has ecological importance due to the hazard at population level considering the cumulative effects of other environmental pollutants.

TU 020
Ecotox findings for ammonium perfluorohexanoate
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Ammonium perfluorooctanoate (APFO) has long been used for polymerization of fluororesins and rubber. There are many reports on the hazards of perfluorooctanoate (C8). Accordingly, its replacement substances have been examined. Perfluorooctanoate (C6) disappears from a rodent's body and perfluorobutyrate (C4) disappears rapidly from rodent's and primate's bodies.
The toxicities of both substances are low. It is considered because they do not accumulate in the body. The accumulation property in the body is considered to have a correlation with the partition coefficients. Accordingly, we examined the correlation between the partition coefficients and effects on Daphnia magna. Also, we studied the effects of C6, which is available for industrial use, on development of fertilized eggs of rainbow trout.
The relation between LogPow of perfluorinated carboxylates (PFCAs=C4, C5, C6, C7, C8) and immobility ratio of Daphnia magna was examined. As the carbon number increased, the LogPow also increased. For C4 and C5, immobility rate became 100% in 24 hours. While C8 reached 100% in 48 hours, the immobility rates of Daphnia magna for C6 and C7 were at the same level. For PFCAs, no correlation between the carbon number and immobility was observed.
In fish early life stage toxicity test of C6, hatching success in the control group was 74%, satisfying the validation criterion for hatching success (>66%). The NOEC and LOEC for hatching success were determined as 10 and >10 mg/L, respectively.
Larval survival until Day 28 post-hatch in the control group was 93% thereby exceeding and satisfying the validity criteria for post-hatch survival (70%). Post-hatch survival across all remaining treatments ranged between 95 and 100%. In terms of concentrations, the NOEC and LOEC for post-hatch larval survival until Day 28 were both considered to be equal to or greater than 10 mg/L.
For fish total lengths, the NOEC and LOEC determined on Day 28 post-hatch were 10 and >10 mg/L respectively. For fish dry weights, the NOEC and LOEC determined on Day 28 post-hatch were 10 and >10 mg/L respectively. All validity criteria were satisfied during the test, therefore the test was considered to be valid. C6 proved to have no impact on fish in an early life stage toxicity test.
Industrially, C6 is easy to produce than C7. It can also be used for polymerization, therefore, it is considered to be a good replacement substance.

TU 021
Determination of perfluorinated compounds in cord blood samples by an on line method : turbulent flow chromatography LC-MS/MS
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The PFCs are persistent compounds that bioaccumulate attached to proteins. Toxicity studies in mammals revealed they can disrupt different metabolic activities. Early exposures have been studied and different authors have reported the transference during breast feeding or during pregnancy. This work presents the preliminary results of a cord blood study in Catalonia (Spain) for 18 PFCs in 165 cord blood samples.
The mixture of PFCs standards used was: MXB (13perfluoroacids and 4perlfuorosulfonates) and perlfuorooctanesulfonamide (PFOSA) from Wellington Laboratories Inc., Canada. Extraction and separation was achieved using a Thermo Scientific Aria TLX-1 system utilizing TurboFlow[TRADEMARK] technology (Thermo Fisher Scientific, Franklin, MA). For the purification process were used 2 TurboFlow columns Cyclone and C18 XL (Thermo Fisher Scientific).
The analysis consists in, internal standard addition, protein precipitation with acetonitrile and centrifugation followed by the analysis using turbulent flow chromatography couplet to tandem mass spectrometry (LC-(QqQ)-MS/MS). The influent solvent consisted in water pH 3.4 (HFo) at turbulent flow of 1.5 ml/min. The loop elution volume of the optimized method was 250µl. Transference was performed using water pH 3.4 (HFo): methanol (20:80) and water pH 3.4 (HFo): methanol (70:30) at flow of 0.2 ml/min. Separation was carried out in a LC-column Hypersil GOLD PFP (50 x 3) (Thermo Scientific). The mobile phase consisted of (A) aqueous ammonium acetate 20 mM (B) MeOH. The total run time for each injection was 16 min at 0.4 mL/min. Thermo Scientific TSQ Vantage mass spectrometer (Thermo Fisher Scientific, San Jose, CA), coupled to TLX-1, was used for analytical purposes, and equipped with a Turbo Ion Spray source.
The preliminary results of this study presents the profile and concentrations of 18 PFCs in cord blood samples from Catalonia (Spain). In this study we found that the more representative compounds were PFOA and PFHxS in agreement with other studies [3]. The concentration levels were 5361-828 ng/L and 10609-536 ng/L for PFOA and PFHxS, respectively. Other relevant compound was PFBA found in higher concentration 53900 ng/L.
The obtained results are in agreement with the literature, however PFOS has been found in lower concentrations than previous studies. This study can provide valuable preliminary information to study the transplacental exposure to perfluorinated compounds.

TU 022
Plastic debris and toxin releases in the Pacific Ocean
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Plastics comprised the majority of collected waste in worldwide beach cleanups in 2006, 2007 and 2008. In California, Washington, Oregon, and Hawaii the five most common plastic debris items on beaches are cigarette filters, food wrappers and containers, beverage caps and lids, bags, and food service items, e.g., cups, plates, and cutlery. The majority of plastic items are made from four common plastics; polyethylene, polypropylene, polystyrene, and PET, accounting for 75% of the plastic debris. Pre-production plastic pellets also account for significant amounts of plastic in the oceans from storm run-off of industrial areas. The fate of plastics in the oceans can lead to fragmentation and result in slurry of plastic particles that can degrade and release toxic chemicals such as phthalates, flame retardants, BPA, antimony oxide, heavy metal inks, and styrene monomer as the plastics break down. Plastics can accumulate toxins floating in the oceans from persistent organic pollutants (POPs). POPs can include DDT, hexachlorobenzene, polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons, among others.

TU 023
Micro-plastics in the marine environment - a global assessment
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GESAMP has set up a new Working Group entitled 'Sources, fate and effects of micro-plastics in the environment - a global perspective'. With multi-agency support and with a multi-disciplinary membership, it is reviewing a wide range of information about the supply of plastics and microplastics to the ocean, and the biological, chemical and physical processess controlling transport, fate and effects of both micro-plastic particles and the contaminant burden. It will provide an evidence base for use by policy mmakers, regional bodies a other stakeholders, and help to develop research priorities. The poster will describe the Terms of Reference, work programme, membership and intended outcomes of the group, and will issue an invitation to SETAC Europe participants to become involved in this initiative

TU 024
Marine micro litter under the marine strategy framework directive - science and policy
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The European Marine Strategy Framework Directive 2008/56/EC (MSFD) recognizes Marine Litter as one of the indicators for the Environmental State of the European Seas. The Commission Decision on criteria and methodological standards on good environmental status (GES) of marine waters (Commission Decision 2010/477/EU), lists specific indicators for assessment of Marine Litter. Indicator 10.1.3 regards marine micro-particles, in particular micro-plastics:
Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source (10.1.1)
"' Trends in the amount of litter in the water column (including floating at the surface) and deposited on the sea- floor, including analysis of its composition, spatial distribution and, where possible, source (10.1.2)
"' Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro- plastics) (10.1.3)
10.2. Impacts of litter on marine life
"' Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis) (10.2.1).
A technical working group under the MSFD implementation strategy has prepared technical recommendations in support of the implementation of the directive by EU Member States. The group identified options and available tools for the monitoring of Marine Litter and developed a roadmap showing the necessary next steps in preparing the implementation of the directive. The poster is presenting specific information regarding marine microlitter under the MSFD.

TU 025
Using the continuous plankton recorder to determine the abundance of microplastic debris in the subsurface marine waters
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Fragmentation of plastics debris into "microplastic"pieces is an emerging issue of concern. Our knowledge of distribution and accumulation of microplastic is patchy and in order to quantify and monitor the distribution and in particular temporal trends there is a need for a broad sampling programme and standardised methods. The aim of this research is to study the spatiotemporal abundance of microplastic debris in ocean surface waters and our preliminary investigation of microplastic contamination in Continuous Plankton Recorder (CPR) samples reported by analysts at the Sir Alistair Hardy Foundation for Ocean Science (SAHFOS) has confirmed the presence of synthetic polymers in several European locations (North Sea, Irish Sea, English Channel and the North Atlantic). We used Fourier Transform Infrared (FT-IR) spectrometry to identify the unknown pieces. The most common plastic types were Polyethylene terephtalate(PET) followed by Nylon and Acrylic but mean abundance was typically less than one item per cubic meter of sea water.

TU 026
Microplastics in personal care products from Brazil: is this source relevant to the aquatic contamination?
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Plastics have been recently incorporate into the group of emerging pollutants due to their wide distribution in marine and coastal environments all over the world, as well as their persistency and hazard to the wildlife. Moreover, they have highly diverse diffuse sources and high capacity of distribution. In addition to other sources, microplastic (< 5mm) have been recently reported in frequently used skin cleansers and others personal care products. As a result, this specific class of plastic has been found contaminating sediments and pelagic zones. Despite the effects related to direct ingestion, microplastic might also transfer adsorbed organic contaminants (OCs) to aquatic organisms. In the current study, particles from 18 of the most frequently used personal care products (soaps and creams) in Brazil were characterized regarding amount , size, form and composition. Analyses were performed by suspension and filtration of plastic particles, followed by microscopic analysis and weight of the particles. Undergoing chemical analyzes by Fourier transform infrared spectroscopy (FTRI) will confirm the apparent predominance of polystyrene among plastics. The amount of plastic ranged from 1.3 to 103.1 mg g^{-1} of product, whereas the distribution frequency ranged from 100 to 400 microns. Due to methodological limitation s, only 10 products had their size measured. Most of the plastics have irregular shapes, although spherical particles were also seen in some products . The preliminary results pointed out this as a relevant source of microplastic to the environment. However, this physic-chemical characterization of personal care products is essential to assess their potential to adsorb, concentrate and transfer OCs, improving somehow their bioavailability, from the environmental to the organisms.

TU 027
International Pellet Watch : background levels, hot spots, legacy pollution, and temporal trends
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International Pellet Watch (IPW) is a volunteer-based global monitoring program designed to monitor the pollution status of the oceans and to understand the risks associated with chemicals in marine plastics. Based on the analysis of resin pellets from remote islands, background levels of persistent organic pollutants (POPs) have been tentatively determined. By comparison of POPs concentrations in pellets from industrially-developing countries (e.g., Ghana) with the background levels, PCB inputs from e-wastes were suggested. Through global comparison, hot spots of PCB pollution were identified, e.g., Le Havre (France), Athens (Greece), Tokyo Bay (Japan), Sydney Harbor (Australia), Los Angeles, San Francisco, Boston Harbor, New York Bight, Lake Erie (USA), Santos Bay (Brazil). Combination of IPW with passive air sampling indicates that legacy pollution is major contributor to most of the hot spots of PCB pollution. In addition to the spatial variation, pellet watch was applied to reveal temporal trend in POPs pollution in California coast and Tokyo Bay.

TU 028
Partitioning between water and plastics of polychlorinated biphenyls in marine animals
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The presence of plastic debris in the oceans is a potential hazard for marine animal species in several ways. It is not only the plastics themselves that form a potential hazard. Chemical additives are added during plastics production in order to catalyze monomers into polymers and give it different properties. Some of these additives have been associated with carcinogenic and endocrine disrupting effects. Moreover, due to its hydrophobic nature, plastic debris can be a hazard to marine animal species because of accumulation of organic contaminants. An important hazard are the persistent organic pollutants (POPs), which do not completely dissolve in water and do not degrade into harmless materials in a short timeframe. Exposure to POPs, e.g. polychlorinated biphenyls (PCBs), has been associated with a wide range of health impacts on both wildlife and humans. This study focuses on the equilibrium partitioning of polychlorinated biphenyls after ingestion by marine animals. In order to get more insight in the bioaccumulation potential of these persistent additives, we compared plastic-water partitioning, a simplification of the partitioning of plastic additives in the gastric fluid, with octanol-water partitioning. The outcomes will be discussed within the framework of the current knowledge gap and we will describe challenges for future research on this topic.

TU 029
Potential for increased exposure to persistent organic pollutants through ingestion of marine debris: the relevance of partitioning coefficients
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An important step in predicting the availability of plastic-sorbed persistant organic pollutants (POPs) to the marine environment is understanding the plastic:water partition coefficient (Kpw) for a great number of chemical and plastic combinations. While plastics have the capacity to absorb chemical substances from their surrounding environment, little is known about the factors effecting sorption and desorption of POPs to plastic debris, the abundance and bioavailability of POPs on plastic debris, and the amount of plastics marine organisms consume. One step in understanding the potential for exposure is an evaluation of relevant partition coefficients.
An extensive literature search was performed for this presentation. This review summarizes articles that calculated and presented plastic to water partitioning coefficients. Only published, peer reviewed literature was examined. The results of our review identified Kpws for 22 specific types of polymers and 198 individual compounds representing 24 different chemical groups. Analyses of these data showed a wide range of values (e.g., 0.91 to 7.8 log Kpw) among compounds for any given polymer. Of note, our correlation analysis showed a relationship between log Kpw and log Kow (octanol-water partition coefficients) for most polymers. Consequently, log Kow may act as a surrogate for estimating log Kpw, which could allow for assessment of availability based on known properties of chemicals and plastics.
This finding is of particular importance because of the lack of log Kpw data for a wide array of plastics. A logical next step is to use log Kow to generate log Kpws through modeling of a wide range of plastics in order to understand the potential for these plastics to adsorb POPs in then environment. It would also be possible to further substantiate these finding by gathering partition coefficients and related information from other sources such as plastics companies, federal/state agencies, etc.

TU 030
Determination of DEHP in culture media by GC-MS/MS using PCI Ammonia
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This method is a mechanism of elucidating the target phthalate, bis (2-ethlyhexl) phthalate (DEHP), in culture media by gas chromatography and tandem mass spectrometry (GC-MS/MS) using positive chemical ionization (PCI) with ammonia reagent gas. Phthalates are routinely analyzed in electron ionization (EI) with 149 m/z as the principal ion. Since all phthalates have similar spectra, it is difficult to identify which phthalate is present. By using PCI, the molecular ion is formed. Culture media samples with varying concentrations of DEHP were analyzed on a GC-MS system consisting of an ion-trap series mass spectrometer and a gas chromatograph. Structural elucidation was achieved by performing MS/MS on the molecular ion. Next, the fragments formed were compared to those in Mass Frontier software, which predicts fragmentation and the mechanisms of product ion formation by comparing the scan results to existing library data. Ammonia was selected as the chemical ionization reagent gas to increase the yield of the molecular ion.
A calibration curve with points at 0.1, 1, 5, and 10 µM were analyzed in culture media. The correlation coefficient for this calibration curve was 0.9969. Two unknown samples were analyzed and quantitated against the calibration curve. These were found to contain 4.2 µM and 3.1 µM DEHP

TU 031
Selective uptake of microplastics by a marine bivalve (Mytilus edulis)
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Microplastics, the degradation product of larger plastic debris, are accumulating in marine habitats worldwide. Given their small size (< 1 mm) these particles can potentially be ingested by marine animals. Data on the uptake of microplastics in invertebrates is, however, scarce. In this study, we tested the hypothesis that the uptake of microplastics from the gut to tissues is size-selective and has an adverse effect on the energy allocation in the mussel.
The blue mussel (*Mytilus edulis*) was exposed for 14 days to three different sizes of microplastics simultaneously (10, 30 and 90 µm). 10 µm-particles, as well as 30 µm-particles, were added at 50 particles.mL^{-1} and 90 µm-particles at 10 particles.mL^{-1}, resulting in a total concentration of 110 particles.mL^{-1}. Analysis of the faeces demonstrated that *M. edulis* filtered and ingested all particle sizes. In the faeces, the 30 and 90 µm-particles were detected in the same ratio as they were added to the seawater (i.e. 5/1). However, the 10 µm-particles were detected in much smaller quantities than expected based on the observations of the other particle sizes, i.e. 8 times less than the 30 µm-particles were observed (even though they were initially present at the same concentration). Although this discrepancy was probably partly due to difficulties in detecting the particles in the faeces, analysis of acid-destructed mussels and hemolymph samples demonstrated that 10 µm-particles were able to translocate to the circulatory system of the mussel. Using a syringe, 300 µL of hemolymph was extracted from the posterior adductor muscle and on average 15.3 ± 3.2 particles (n=15) were found in the hemolymph of the mussels. This uptake accounts for 0.3% of all 10 µm-particles ingested. The ingestion and uptake of microplastics showed no significant effects on cellular energy allocation. Exposed mussels did show a significant increases in energy consumption, indicating a rise in metabolic activity.
Although *M. edulis* was exposed to a very high concentration of microplastics, which was aproximately 1000 times the environmentally relevant concentration, no significant short-term adverse effects of ingestion and translocation were detected. Long-term exposure studies could, however, provide more conclusive answers on the effects of ingestion and uptake of microplastics in *Mytilus edulis*.

TU 032
Presence of plastic debris in sea turtles stranded along the Tuscany coasts
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Marine debris is proven to have a widespread negative impact on marine wildlife, including physiology-level effects and reduced fitness, reproductive failure, changes in community structure to direct death following blockage of intestinal tract or entanglement.
The main sources of marine debris are litter from ships, fishing and recreational boats, and garbage carried into the sea from land-based sources in industrialized and highly populated areas. Marine debris is an important threat for sea turtles and the evaluation of the impact of plastics on development, survivorship, health and reproduction of sea turtles is highlighted as one of the global research priorities for these threatened species. The loggerhead turtle (*Caretta caretta*) and leatherback turtle (*Dermochelys coriacea*) are considered two of the sea turtles species ingesting the largest amount of debris, in all their life stages, most likely because of their habitat and feeding behaviour.
In this study we quantified plastic debris ingestion in twenty-nine specimens of loggerhead turtle having a curved carapace length (CCL) of 29.0-71.0 cm. Moreover we had the opportunity of collecting one specimen of leatherback turtle incidentally captured dead by fisheries in Elba Island (CCL of 124.0 cm).
During dissection of animals, biometric parameters were taken and the entire gastrointestinal tract was removed and subdivided into oesophagus, stomach and intestine. In a second step the debris and litter were collected from oesophagus, stomach and intestine and the plastic founded were subdivided in different categories: sheetlike, threadlike, foamed and fragment user plastics. Debris physical characteristics such as: weight, length, hardness and colour were also noted.
The first result obtained from this study was that a large number of plastic was found, prevalently into the intestine, in more than 60% of the animals. The main categories of plastics found in the specimens were soft sheetlike user plastics, that the animals can mistake to jellyfish. This may support the assumption that loggerheads are attracted by floating debris more than by debris on the sea floor. The second type of plastics found in the specimens were fragments; moreover in some specimens we found little amounts of tar in the entire gastrointestinal tract. Another important result obtained was the absence of food in the specimens that show an high number of plastic debris.

EP08P - What is the current state of the science on the fate, exposure and effects of pharmaceuticals in the environment?

TU 033
Occurrence of Triclosan in fresh waters from São Paulo, Brazil - the need for regulatory actions
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Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)phenol, is a broad-spectrum bactericide used in pharmaceuticals and personal care products of daily use. It is a non-volatile compound (5.3 x 10-4 Pa at 20 oC), relatively soluble in water (10 mg /L at 20 oC) with a log Kow of 4.8. Triclosan is highly toxic to aquatic organisms and some PNECs were proposed in the literature, varying from 1.4 to 1550 ng/L.[1] In Brazil triclosan is not regulated and there aren't reports about the presence of triclosan in surface waters. In this work, six rivers from the State of São Paulo (Atibaia, Capivari, Sorocaba, Cotia, Tanque Grande and Rio Preto) were monitored for triclosan in a one year study. Caffeine was monitored as a surrogate of domestic source of contamination. One liter of water samples was filtered using 0.45 µm pore size cellulose acetate membranes, extracted by solid-phase extraction and the compounds were determined by liquid chromatography tandem mass spectrometry. The limit of quantification (LOQ) of method applied were 0.7 ng/L for triclosan and 15 ng/L for caffeine. From 100 samples analyzed 43% presented triclosan at concentrations ranging from 2.2 to 66 ng/L, and 98% presented caffeine at concentrations about 1,000 times higher than triclosan, but no correlation was found with triclosan concentrations. No differences were observed in dry or wet seasons. If we compare the results with the lowest PNEC found in the literature the 43% of the samples where triclosan was found the concentrations were above 1.4 ng/L. If we using the PNEC value 4,7 ng/L, 40% of the samples presented concentrations above that limit. And even If we apply PNEC of 50, 3 samples were determined triclosan concentrations above this amount. Our results suggest that triclosan is an ubiquitous substance and should be considered a priority pollutant at least in the State of São Paulo and therefore included in the water regulation in order to protect the water for aquatic life. Further studies in other water bodies in Brazil are needed to determine the ocurrence of triclosan nationwide.

TU 034
First pilot study on the occurrence of UV screens and preservatives in a Victorian estuary
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The southern hemisphere's elevated UV light exposure encourages the year-round use of UV protective chemicals (UV filters and UV light stabilizers) in personal care and plastic products. Some UV-filters and preservatives are known to be hormonally active in vitro and/or in vivo, raising concerns of possible endocrine disrupting effects in the environment. There is little published data of occurrence of those compounds in Australian aquatic environments. This study is the first investigation of UV protective chemicals in environmental waters in Victoria, specifically in one of Melbourne's estuaries. Water samples were taken from four sites (A - D). Screened compounds included 11 UV filters, 10 UV stabilizers, 12 preservatives and the metabolite, and one fragrance, including commonly used compounds in Australia such as 4-MBC, EHMC, octocrylene, UV-328, HHCB, 2-phenoxy ethanol, methyl paraben, and propyl paraben. Water samples were extracted for polar and relatively non-polar compouds using two different types of solid phase extraction methods: Oasis HLB (Waters Corp.) for polar compounds, Empore C18FF (3M) for relatively non-polar compounds, then anlysed by GC-MS, after derivatization for polar compounds. Samples from Sites A, B, and C had low concentrations of the screened chemicals with similar compound profiles. Site D had a very different contaminant profile, with very high concentrations of many of the screened compounds. Sites A, B, C are both at the mouth of creeks and rivers flowing into Port Phillip Bay; Site D is about 5 km upstream of site C in Melbourne on the second biggest river entering Port Philip Bay. Although there are several small wastewater treatments located up stream of site D, the levels of HHCB (an indicator of domestic and municipal wastewaters) measured at Site D were similar to the levels observed at Sites A, B and C, suggesting that the chemicals observed at site D do not originate from a wastewater treatment plant but a different source. The detection of common UV filters, such as 4MBC, EHMC, OC and the common preservatives 2-PE, MP, and PB in a Victorian estuary proves that the existence of personal care products in the environment is not just an issue for more densley populated countries in the northern hemisphere, but also potentially of concern in Australia.

TU 035
A comprehensive study of pharmaceuticals sources and fate in a highly urbanized and inhabited area in Italy
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Pharmaceuticals are used in high quantities in our society. They can be excreted unchanged and/or as active metabolites in urine and feces, and direclty conveyed to wastewater treatment plants (WWTPs). If they escape degradation during wastewater treatment, they finally end up in surface water. The present study aimed for the first time to study sources and fate of several selected classes of pharmaceuticals in the most inhabited and industrialized area in Italy (urban and suburban area around Milan). The investigation was conducted in waste, surface and ground water. Composite samples were collected, filtered, enriched with internal standards and solid-phase extracted. LC-ESI-MS/MS with multiple-reaction monitoring and stable-isotope dilution assay was used for quantitation. Recoveries were generally higher than 80% and limits of quantifications were in the low ng/L range. Ciprofloxacin, ofloxacin, atenolol, furosemide, hydrochlorothiazide, clarythromycin, ibuprofen, ketoprofene, diclofenac, naproxen, and carbamazepine were the most abundant residual drugs in urban wastewater. Their removal in conventional WWTPs was generally lower than 50%, with no removal for bezafibrate, hydrochlorothiazide, furosemide and carbamazepine. Removal rates were also affected by the type of advanced treatments adopted (i.e. disinfection, UV treatment) in the plants investigated. The amounts of pharmaceutical active substances discharged in the environment daily through treated wastewater ranged between 1.2 and 2 kg. The total amounts of the investigated pharmaceuticals in the rivers flowing though the area investigated ranged between 0.9 and 2.7 kg/day respectively before and after the city of Milan. Traces of some compounds as carbamazepine were found in the low ng/L range also in untreated ground water. Moreover, the pattern of contamination of ground water was higher in sites closer to rivers, suggesting the contribution of surface water to the contamination. This investigation allows the quantification of the mass flow of these substances flowing through a highly urbanized and industrialized area.

TU 036
An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants
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Increased attention is currently directed towards potential negative effects of pharmaceuticals and other micro-pollutants discharged into the aquatic environment via municipal sewage water. A number of additional treatment technologies, such as ozonation, have therefore been suggested as promising tools for improving the removal efficiency of pharmaceuticals in existing Sewage Treatment Plants (STPs). Constructed wetlands are also capable of removing a variety of micro-pollutants, including some pharmaceuticals, and could hence be a resource efficient complement to more advanced treatment technologies. The purpose of the present study was therefore to increase the knowledge base concerning the potential use of constructed wetlands as a treatment step to reduce emissions of organic micro-pollutants from municipal sewage effluents. Under cold winter conditions, incoming and outgoing waters from four Swedish free water surface wetlands, operated as final treatment steps of sewage effluent from municipal STPs, were sampled and analyzed for levels of a set of 92 pharmaceuticals and 22 inorganic components as well as assessed using subchronic ecotoxicity tests with a macro alga and a crustacean.
Sixty-five pharmaceuticals were detected in the range 1 ng L^{-1} to 7.6 µg L^{-1} in incoming and outgoing waters from the four investigated wetlands. Although the sampling design used in the present study lacks the robustness of volume proportional 24 h composite samples, the average estimated removal rates ranged from 42 to 52%, which correlates to previous published values. The effects observed in the ecotoxicity tests with the macro alga (EC50s in the range of 7.5 to 46%) and the crustacean (LOECs in the range of 11.25 to 90%) could not be assigned to either pharmaceutical residues or metals, but in general showed that these treatment facilities release water with a relatively low toxic potential, comparable to water that have been treated with advanced tertiary treatments.
From the present study it can be concluded that constructed wetlands may provide a complementary sewage treatment option, especially where other treatment is lacking today. To fully remove micro-pollutants from sewage effluent however, other more advanced treatment technologies are likely needed.

TU 037
Large screening of pharmaceuticals and hormones in sludge based on LC-ToF-MS
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Ecosystems are potentially contaminated by many chemical substances from domestic activities, through the spreading of treated sewage sludge. Indeed during the last thirty years, the application of municipal sludge from urban to agricultural soils is the option favoured internationally for sludge management as it contributes positively to recycling nutrients, soil properties and fertility. The literature data indicate that the removal efficiencies of organic contaminants in sewage treatment plants vary between 24 and 100% depending on the contaminants and treatment processes, which means that significant percentages of products can be present in the sludge.
Steroid sex hormones and pharmaceuticals are considered emerging environmental contaminants of particular concern, as many of them display biologically active properties (including endocrine-disrupting). At the European level, few studies exist on the characterization of these emerging substances in sludge or on the assessment of health and environmental risks associated with their use.
This is partly explained by the difficulties of detection and quantification of such pollutants in the solid phases. It is, indeed, a real analytic challenge given the diversity of analytes, their presence generally in trace amounts and the complexity of the matrix which makes difficult the extraction step. Thus it appears that the development of analytical methods sensitive, reliable and robust is an essential step for the characterization of emerging substances in sludge.
In this context, the aim of the study was to develop a method for the large screening of pharmaceuticals and hormonal steroids in sludge. Therefore, nearly two hundred substances were analyzed by liquid chromatography-time-of-flight mass spectrometry. The analysis of complex matrices such as sludge needed a rigorous sample preparation to obtain a repeatable and enough sensitive analysis to achieve the detection limits required. For this purpose, an extraction step using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was set up. This analytical methodology was successfully applied to various kinds of sludge (limed, digested, dried, liquid, composted) collected in several sewage works in France.

TU 038
Adsorption of human pharmaceuticals to activated sludge: how many different sludges should be tested for an environmental risk assessment?
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The potential risk of human pharmaceuticals to the environment has to be determined, e.g. according to the EMEA Guideline EMEA/CHMP/SWP/4447/00. This guideline focus on the risk by the use of the pharmaceutical and the entry into the environment via excretion and wastewater effluent to a sewage treatment plant (STP). It is assumed that a substance with high sorption to activated sludge may reach terrestrial ecosystems by land spreading of sewage sludges. The adsorption to sludge has therefore to be tested according to the EMEA Guideline in a adsorption study. A terrestrial risk assessment must be additionally conducted with the drug if its adsorption coefficient to sludge exceeds a defined trigger value (Koc of 10'000 L/kg or Kd of 3'700 L/kg) and the drug is not readily biodegradable.
The sorption of chemicals to sorbents like soils or sediments typically depends on several parameters of the sorbent (e.g. pH, ion-exchange capacity, redox potential, organic carbon content, clay content, texture). The question arises how different activated sludges are regarding these parameters, how large the adsorption to sludges varies and consequently how many sludges should be tested for a safe risk assessment. The number of sludges to be tested is not clearly defined in the guideline EMEA/CHMP/SWP/4447/00.
In this presentation the results of batch equilibrium sorption tests with different sludges will be shown for several pharmaceuticals. The variability of the adsorption coefficients, obtained for different sludges by the same analytical method and within the same laboratory will be discussed in the context of the trigger values used in the environmental risk assessment.

TU 039
Analysis of antimalarial drugs in water
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Malaria is a general illness in many countries and therefore antimalarial drugs are prescribed in great quantities. There are more than 20 different molecules being used

for treatments. These drugs are excreted mainly via urine. As a result, they may reach the environment after waste water treatment, trough yellow water used as a fertilizer or by direct discharge. There is limited information on the fate of antimalarials in the environment. It is believed that some of these drugs are rather persistent due to their organohalogen moieties. Hence, the fate of these drugs in the environment might be a subject of concern.
To date, many methods to analyze antimalarial drugs in blood and urine been published. However, only two methods have been published to analyze a few antimalarial drugs (artemisinin derivates and chloroquine) in water or soil. For that reason, the first multi-residue method for the analysis of antimalarial drugs and their metabolites in water has been developed. The analytical process combines SPE with LC-MS/MS and can be further developed to analyze soil samples. The presented method can be used to describe environmental fate profiles of antimalarial drugs in the environment.

TU 040
Matching micropollutant loads of influent and effluent for reliable mass balances in WWTPs
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The removal efficiency of pollutants such as COD in a WWTP is usually evaluated by data from long-term time series where variation caused by transient flow and concentration are averaged out. The analyses of micropollutants is however work and cost-intensive and that is why measurement campaigns usually only cover short periods with a small number of samples available to establish a mass balance. Considering that biological reactor tanks are well-mixed systems, a sampling scheme comprising single 24-h composite samples in inlet and outlet is most likely to compare two largely different water volumes. For a completely-mixed system, it was shown that a 24-h effluent load is composed of water volume fractions from several previous days (Majewsky et al., 2011).
In the present study, we designed an individual sampling strategy using hydraulic calibration of a Luxembourg WWTP to validate the proposed model. The elimination efficiencies of 5 pharmaceuticals were calculated by a 3-day influent and 1-effluent campaign on the basis of 2-h composite samples (time-proportional) which allowed to explain >80% of the origin of the effluent load sampled. Results showed that the method was capable of reproducing realistic elimination values while short-term mass balances lead to erroneous values.
The validated model allowed to evaluate the accuracy of different sampling scenarios as a function of the sampling mode and frequency (Ort et al., 2010) and of the number of samples taken in the inlet and outlet of the WWTP. The aim is to maximally reduce the uncertainty and/or to explain the bulk of the effluent load origin. A relationship can be established between the influent proportions that are captured by an effluent sampling period and the mean hydraulic retention time (HRT). Based on these findings, we propose to develop a generic method to design sampling strategies in WWTPs without having to rely on prior hydraulic calibration.
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TU 041
Occurrence and fate of triclosan and triclocarban during wastewater and biosolid treatment
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Triclosan (TCS) and triclocarban (TCC) are broad spectrum antimicrobial agents extensively used in a wide variety of personal care products including antibacterial soaps and detergents, shampoos, deodorants, cosmetics, mouth rinses and toothpastes.
During wastewater treatment, the removal efficiency of triclosan and triclocarban from the aqueous phase can be considerable in activated sludge processes but is dependent on treatment techniques. Triclocarban is recalcitrant during the sewage treatment process and is detected in streams and sediments downstream of WWTPs. Triclosan is amenable to biodegradation during sewage treatment and both TCS and the transformation product methyl-triclosan (MTCS) are detected in WWTP effluents.
TCS, TCC and MTCS have lipophilic properties and hence partition to sludge during wastewater treatment and may be present in concentrations ranging from ng kg-1 to % in the dry solids depending on the initial amounts present and the extent of removal during wastewater and sludge treatment. Moreover, the sludge accumulated WWTPs is often processed to produce biosolids intended for land application. Thus, the agricultural recycling of biosolids, the favored option for sludge management, may lead to adverse environmental impact.
In this work, TCS, TCC and MTS were monitored in both waters and sludge at different stages within the wastewater treatment process in two WWTPs with different characteristics located in the coastal area of Girona (NE Spain). Moreover, the influence of sludge digestion process on the degradation of target compounds was evaluated by analyzing the resulting digested biosolids under both aerobic and anaerobic conditions. The effect of the temperature on the occurrence of the studied compounds in the final biosolid was also investigated.

TU 042
Dominant time scale of antibiotics concentrations in waste waters through one-year field campaign study
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Among the long list of pharmaceuticals that can be found in the aquatic environment, antibiotics receive important and increasing attention, as they are suspected to foster bacterial resistance. But if many studies have listed their constant presence in waste waters, few of them curiously have focused on the temporal dynamics of their concentrations. In the presented study, we compare the relative importance of monthly, weekly, daily, and hourly time scale when assessing the dynamics of antibiotics in waste waters. For this, a one-year experimental sampling campaign was conducted at the Vidy WWTP of Lausanne in Switzerland. One week a month during one year, hourly samples were taken from WWTP influent. Samples were aggregated together to obtain measured hourly variations, daily variations, monthly variations and seasonal variations. A total of more than 180 samples over year 2011 were analyzed using UPLC-MS/MS, and the concentrations of 9 antibiotics (azythromycin, ciprofloxacin, ofloxacin, clarithromycin, clindamycin, metronidazol, norfloxacin, sulfamethoxazol, trimetoprim) compared. We conclude that even if a seasonal trend for concentrations can be observed for several substances, the hourly time scale totally dominates temporal fluctuations of concentrations. In other words, there is a high hourly fluctuation of antibiotics concentrations, which is superior in magnitude to other (daily or seasonal) time scales. Thus, more efforts in terms of modelling and treatment optimization should be conducted at this temporal resolution (hour) for antibiotics, in order to reduce the risk they can represent for the environment.

TU 043
Quantification of pharmaceuticals and endocrine disruptors in river sediments: development and validation of a QuEChERS based extraction
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Accessing exposure concentrations can be challenging for the analyst as environmental concentrations and toxicological or eco-toxicological effect induced quantities are often not consistent with analysis capacities. To encounter those limitations analysts use pre-concentration and purification steps like Accelerated Solvent Extraction (ASE) and Solid Phase Extraction (SPE) to extract and analyse pharmaceuticals or hormones at the lower or sub ng/g level from solid matrices. A recent extraction method known as Quick Easy Cheap Effective Rugged and Safe has been developed for the analysis of pesticides in food matrices.
This method has already been extended to various matrices like rats' serum and gonads [1] in our laboratory. We developed and validated a multiresidue analytical method with QuEChERS extraction for the screening of multi-families of pharmaceuticals and endocrine disruptors in river sediments. The molecules were chosen among the priority pollutants listed in a prioritisation for French surface waters [2], in the Water Framework Directive and within the potential or confirmed EDCs. This selection of 13 pharmaceuticals includes β-blockers, anti-inflammatories, antibiotics, anxiolitics, anti-depressants and anti-analgesics 24 EDCs includes pesticides, alkylphenols, hormones and phenolic derivates and. Quantification is performed on a 3200QTrap tandem mass spectrometer (MS/MS) coupled to Liquid Chromatography (LC) Agilent 1200 system with a Kinetex XB-C18 column (100x2.1mm, 1.7µm).
Extraction was optimised to get acceptable recoveries (>60%) and the linearity of the method has been verified by statistical means. Acceptable Relative Standard Deviations of both injection and preparation steps (<20 to 30%) were calculated for intraday injections of 3 independent solutions. Repetition of a calibration over three days allowed us to calculate good interday RSD. Limits of quantification were determined by injection of 5 independent standard solutions and were consistent with environmental concentrations.

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TU 044
Developing a miniaturized push-pull test to study the transformation of pharmaceuticals in the hyporheic zone of rivers
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The hyporheic zone is a key compartment for the attenuation of organic micropollutants in rivers. The comparatively slow flow velocity, the continuous import of nutrients and oxygen, and the export of metabolic waste provide ideal conditions for microbial transformation processes. A better characterization of hyporheic processes would substantially improve the quantification of the self-purification capacity of streams. Unfortunately, measuring transformation rates of organic micropollutants directly in the hyporheic zone is difficult due to limited accessibility, but also due to a lack of suitable experimental methods. Therefore, we are developing a miniaturized push-pull methodology that allows measuring transformation rates of pharmaceuticals directly in the hyporheic zone.
Push-pull tests are an established means of testing the transformation capacity of aquifers with respect to organic or inorganic contaminants. There, a solution containing a set of conservative and reactive tracers is injected ("push") into an aquifer, and after a certain time the groundwater is extracted ("pull") through the same well. The difference between conservative and reactive tracer recovery can then be used to estimate in-situ transformation rates. The aim of our study is to downscale this approach so that it can

be used on the spatial scale of centimetres to decimetres.
The experimental concept is validated in a small flume that allows the simulation of different hydraulic conditions in the hyporheic zone. Under abiotic conditions, we applied the approach successfully to measure retention of several pharmaceuticals at residence times of up to 16 hours; longer residence times will be evaluated. Currently, we are testing the approach under biotic conditions with the same set of pharmaceuticals and, in addition, with some reference compounds. Our contribution will summarize the experimental concept and the major finding of this method development study, and it will highlight the potential of the method for improving our understanding of hyporheic processes and thus of the environmental fate of organic micropollutants.

TU 045
Comparative analysis of contaminant of emerging concern discharges from a centralized municipal treatment plant and on-site wastewater treatment systems receiving common wastewater influent
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Though over 25% of the United States population employs decentralized on-site technologies for wastewater treatment, a comparative understanding of treatment efficacies of these systems remain less understood than those from centralized municipal wastewater treatment plants. In fact, it is estimated that 10 to 20 percent of these systems malfunction annually, releasing untreated wastewater to groundwater and surface water, which can degrade water quality and result in impacts on surface waters in regions experiencing high groundwater - surface water exchange. Even less understood is a comparative understanding of contaminants of emerging concern (CECs) associated with these systems and subsequent loadings to the environment. The primary objective of this study was to assess the occurrence and removal efficiencies of select CECs among several different wastewater treatment systems. Our secondary objective was to evaluate the influences of seasons and influent dosing scenarios on-site aerobic treatment systems. The current study further examined the occurrence of CECs, including drug of abuse metabolites, between weekend and weekday sampling events. The studied CECs covered a variety of common pharmaceutical classes, including analgesic, anti-hypertension, antibiotic, psychostimulant metabolites, antihistamine, anti-seizure, benzodiazepine, anticoagulant, anti-inflammatory, antilipemic, caffeine, and the artificial sweetener sucralose, which appears to represent a robust tracer of anthropogenic activities. An isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) method that employed independent isotopically-labeled standards for quantitation of each compound was applied to quantitate target analytes for all samples. ANOVA was performed to test differences of treatment type, and season on CEC concentrations and other routine water quality parameters. Results showed that concentrations of CECs are typically in the range of <1-100 ng/L in the effluent discharges, which are generally consistent with peer-reviewed literature reports for these CECs. CEC concentrations from advanced on-site systems were reduced relative to septic systems. The present study, which included CECs with varied physicochemical properties, provides an initial understanding of the range of environmental loadings from common on-site wastewater treatment systems.

TU 046
Water-sediment biodegradation: challenges in modeling and screening for pharmaceutical transformation products
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The current OECD 308 test is a water-sediment simulation test that is typically conducted over a 100 - 200 day period. It is intended to estimate the parent half life in the water phase, sediment phase and collectively in the total water-sediment system; determine the distribution and mass balance of the residues in the test system; as well as characterize the potential transformation products of the parent substance over the study period. Given the length of the test period, the analytical expertise and costs associated with conducting the test, it is often desired that a more rapid screen for assessing the potential transformation of pharmaceuticals would be available. Such information would be helpful in developing the ERA testing strategy especially when the identification of a key transformation product would be helpful early on in the risk assessment; as well as optimizing sampling intervals and conditions for the OECD 308. This presentation looks at the observed transformation products identified in the OECD 308 study and compares those to what may be predicted in an expert system to understand how selective the predictions may be and what additional information might be helpful in making a predicted pathway more realistic. Challenges in screening for water-sediment transformations in a laboratory test are also discussed to assess how such a test might be performed. For both situations, case studies will be presented to highlight some of the challenges present in advancing these further.

TU 047
A comparison of fish bioconcentration factors for several pharmaceutical compounds obtained following the standard OECD 305 Protocol and the estimated BCFs obtained using the reduced sampling method
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Understanding whether an active pharmaceutical ingredient is likely to be taken up from the aquatic environment by fish and bioaccumulate has triggered the need to conduct bioconcentration studies in fish. Such studies are typically considered appropriate for pharmaceuticals having a logD value > 3 and required, as per the EMA Guideline, for pharmaceuticals having logD values > 4.5.
The standard OECD Guideline 305 includes an exposure (uptake) phase followed by a post-exposure (depuration) phase typically equal to ½ the duration of the uptake phase. During the uptake phase, ranging between 14 and 60 days, water and fish samples are analyzed periodically until steady state has been established and the depuration phase may be initiated. During the depuration phase, analysis of water and fish samples continues until a plateau has been reached. Based on the sampling schedule outlined in the guideline, >100 fish per study may be required to determine a kinetic bioconcentration factor (BCF). In a effort to reduce the number of fish required to obtain a BCF, an approach to estimating the aquatic bioconcentration factor using reduced sampling has been developed (Springer, et al.2008). This reduced sampling approach aims to estimate BCFs using a minimum number of sampling time points and therefore a reduced number of fish.
The goal of this project is compare the BCFs of several active pharmaceuticals obtained through standard OECD 305 test methods with those BCFs determined using the approach of estimating aquatic bioconcentration factors using the reduced sampling method.

TU 048
Waterborne beclomethasone diproprionate affects fish while beclomethasone is not taken up
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Glucocorticoids are steroid hormones, playing important roles in several aspects of vertebrate physiology. The synthetic corticosteroid beclomethasone has anti-inflammatory actions and is commonly used in the treatment of asthma. It is administered as the more lipophilic prodrug beclomethasone diproprionate (BDP) which is metabolized to the monoproprionate (BMP) and free beclomethasone in humans. A recent publication demonstrated effects of waterborn BDP (1 µg/L nominal concentration) on plasma glucose levels in fish. Although levels in of beclomethasone and its lipid esters in effluents or surface waters are not known, it can be assumed that most of the consumed prodrug BDP has been metabolized into the less lipophilic forms before reaching the environment. We have therefore compared the potential of beclomethasone and BDP to bioconcentrate and to affect the physiology of exposed fish. Controlled flow-through studies with rainbow trout over two weeks to up to 0.65 µg of beclomethasone/L did not result in measurable plasma levels. Exposure to 0.65 µg/L BDP, on the other hand, led to accumulation of both beclomethasone, BMP and BDP in plasma, indicating a considerably more efficient uptake of the more lipophilic prodrug as well as metabolism within the fish. Accordingly, exposure to 0.65 µg/L of BDP significantly increased blood glucose levels, in agreement with previously published results. Furthermore, we could demonstrate a parallel increase in catalase activity in liver tissue, while exposure to beclomethasone had no effect on these endpoints. We are currently screening for beclomethasone, BDP and BMP in sewage effluents and surface water.

TU 049
The degradation of antidepressant pharmaceuticals in aerobic sludge
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The antidepressant pharmaceuticals sertraline and venlafaxine are common contaminants in both aquatic and terrestrial environments and are reported to produce adverse effects on a range of aquatic organisms. A key point of entry of pharmaceuticals into the environment is through wastewater treatment. To learn whether microbial communities in wastewater sludge have the ability to degrade sertraline and venlafaxine and, thus, consequently reduce their environmental loads, microcosms containing aerobic sludge were spiked with deuterated sertraline and venlafaxine. Samples collected over 36 days were analyzed for the depletion of the added pharmaceuticals by microscale solvent extraction followed by liquid chromatography tandem mass spectrometry (LC/MS/MS). Municipal aerobic wastewater sludge was found to degrade sertraline and venlafaxine by over 90% over the course of 36 days, whereas no change in the added sertraline and venlafaxine concentrations were observed in abiotic control microcosms.

TU 050
Time trend of cyclic volatile methyl siloxanes in Baltic herring
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Cyclic volatile methyl siloxanes (cVMS) were studied in herring from the Swedish environmental sample bank. Fish from a site from the Baltic Proper southeast of Stockholm (Landsort) and from the northern part of the Bothnian Bay (Harufjärden) were analysed. Skinless dorsal muscle samples from six individuals were pooled. Two pooled samples were analysed from each site for every second year from the period 1989 - 2009. Octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) were analyzed. The D5 concentrations were of the 50 ng/g lipid weight, while the concentrations of D4 and D6 were lower and frequently below the MDL. The concentrations were generally lower at the beginning of the period and highest around 2005-2007. There were no marked differences in concentration between the two locations.

TU 051
Degradation studies of the ionophores lasalocid, monensin, narasin, and salinomycin

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Coccidiostats are antimicrobial feed additives used prophylactic in poultry production to prevent the parasitic disease coccidiosis. The most heavily used sub-group of coccidiostats are the antibiotic ionophores. The excretion rate of coccidiostats from chickens is high and typically the chicken dung is applied onto agricultural fields. Hence, the aim of this study is to describe the environmental fate of ionophores and to identify new and ecotoxicological relevant transformation products.
Abiotic and biotic transformation studies were conducted on four ionophoric anticoccidials; lasalocid, monensin, narasin, and salinomycin. Abiotic studies focusing on hydrolysis and photolysis were conducted following international OECD guidelines. Hydrolysis was observed under acidic conditions for narasin, monensin and salinomycin, while lasalocid seemed stable under all conditions. Only lasalocid absorbs environmentally realistic wavelengths, so that photo-degradation of monensin, narasin and salinomycin can be discarded. Biotic degradation studies were conducted in lab-scale bioreactors inoculated with Nycodenz extracted soil bacterial communities. In addition, during the work with these very lipophilic anticoccidial agents, it was observed that adsorption to analytical equipment was highly relevant to assess under acidic conditions.

After abiotic transformation of the ionophores, the soil bacterium Nitrosomonas europea was used as a screening tool for transformation product toxicity. This effect-directed study was conducted by exposing bacteria to the mixture of ionophore degradates.

TU 052
Formation and structure elucidation of stable transformation products of pharmaceuticals in the water cycle
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Pharmaceutically active substances undergo transformations starting from human metabolism to degradation in advanced effluent treatment, in environmental processes and finally during drinking water treatment. Often degradation in sewage and water treatment and the environment is incomplete, resulting in the formation of stable transformation products. The formation and presence of such stable transformation products in the effluent of sewage works, surface water, and drinking water treatment is reported in the scientific literature with increasing frequency. In only a few cases, full mineralization of the parent compounds is achieved. This is even more of importance as advanced oxidation techniques employing e.g. ozone, hydrogen peroxide, light or electro-coagulation are increasingly under discussion for the removal of pharmaceuticals and other micro pollutants in effluent treatment and drinking water treatment. Treatments using these techniques may even lead to the formation of transformation products that are more toxic than the parent compound.
In the past years, comprehensive information on the degree of contamination of our environment with pharmaceuticals has been collected, but there are still some important knowledge gaps regarding the ecological and human health impact of two groups of pharmaceuticals, which will be addressed in the Pharmas project (EU grant agreement no. 265346). On the one hand, for anti-cancer drugs only little information is available on environmental concentrations, while for the antibiotics, concern is raised regarding their occurrence and the potential role of these compounds in spreading resistance against these valuable pharmaceuticals.

For a selection of pharmaceuticals of both classes, the formation of stable transformation products in various stages of the water cycle (both drinking and sewage) will be investigated in laboratory and pilot scale studies. Treatment processes include mainly chlorination, ozonation and UV-disinfection for drinking water treatment and advanced oxidation, photolysis/photocatalysis/photo-fenton for sewage treatment. For structure elucidation of the transformation products formed, different LC-MS/MS approaches as well as high resolution MS techniques will be implemented. Characterization of the stable transformation products in terms of (eco)toxicological behavior will be done using a battery of in vitro bioassays.

TU 053
Predicting environmental concentrations of carbamazepine and oxcarbazepine and their main metabolites in a coastal system
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Predicting environmental concentrations of carbamazepine and oxcarbazepine and their main metabolites in a coastal system
Predicting of environmental concentrations of pharmaceuticals is commonly done for risk assessment at a regional level. The difficulties appear for the prediction at a local level, where data available is scarce. This work developed a model to predict the behavior of carbamazepine (CBZ), oxcarbazepine (OxCz) and their main metabolites in a regional environmental system : a coastal zone with a WWTP submarine outfall. The model takes into account the initial CBZ and OxCz prescriptions, metabolisms and partial transformations of CBZ and OxCz into their metabolites, their fate in wastewater treatment plants and their flow to the coastal zone through the submarine outfall. Regional data on CBZ and OxCz prescription were obtained from the medical care system. CBZ and OxCz prescribed amounts have been studied over 6 months in view to calculate concentrations in wastewater treatment plant effluents and so allow for Predicted Environmental Concentrations (PECs) estimation. The human metabolic pathways of CBZ and OxCz required to include in the model the contribution of the major urinary metabolites: carbamazepine-10,11-epoxide, 10,11-dihydro-10,11-trans-dihydroxy-carbamazepine, 10-hydroxy-carbamazepine and other hydroxylated derivatives. PECs lie in the ng/L level in the wastewater effluents and were compared to the measured concentrations (MECs). The diffusion in the coastal zone was estimated with an hydrodynamic numeric model (MARS 3D). The behavior in the coastal zone was qualitatively compared to measured concentrations obtained with POCIS passive samplers. Data on pharmaceuticals in the coastal zone are scare and PECs and diffusion model might therefore be useful for studying pharmaceutical transfer and fate in the coastal environment.

TU 054
Fate of anti- diabetic drugs: gliclazide, glibenclamide and glimepiride in soil environment
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It is assumed that about 3-5% of human population suffers from diabetes mellitus type 2. Since large quantities of anti-diabetic drugs are used for treatment, it is possible that huge amounts of these pharmaceuticals and their metabolites are deposited in the environment due to excretion from humans and because of the improper utilization of off-date drugs. The most common way to enter the natural environment by these drugs is by sewage. Therefore, the most endangered are natural waters, soils and sediments. Considering the composition of these matrices it is obvious that the most dominant interaction are sorption and biodegradation processes. Transport of the compound in porous media, such as soils, sediments and aquifers, is strongly influenced by its tendency to sorb on to the various components of the solid matrix. Furthermore, since molecular transfer is a prerequisite for the uptake of organic pollutants by organisms, the bioavailability of a given compound and thus its rate of biotransformation, is affected by sorption as well.
Sorption process of three sulfonylurea based of anti-diabetic drugs were tested on three various soil. Selected soils differed in their physical and chemical properties and thus, it was possible to assess the influence on sorption of: organic matter content; pH; moisture; fine fraction content and cation/ anion exchange capacities. Other external factors like: change of pH, ionic strength of solution and temperature were also tested. Various of sorption and kinetics models were used to describe obtained results.

TU 055
Evaluation of pharmaceuticals and personal care products focused on anthelmintics in human-waste, sewage, hospital wastewater, livestock wastewater and receiving water
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We investigated 33 PPCPs focused on anthelmintics in the human-waste treatment plants (HTPs), sewage treatment plants (STPs), hospital wastewater treatment plants (HWTPs), rivers and seawater. Additionally, the livestock wastewater treatment plants (LWTPs) were surveyed to assess the concentrations of anthelmintics. The LWTPs and HTPs had relatively high levels, indicating that livestock wastewater and human-waste are principal sources of anthelmintics. In the sources except for the LWTPs, human anthelmintics, albendazole and flubendazole, were the most dominant. In some receiving water samples, veterinary anthelmintics showed higher proportion than human drugs, suggesting the possibility of other sources flowing into the water environment. Overall, anthelmintics showed insignificant removal efficiencies in wastewater treatment plants. Compared with NSAIDs, antibiotics and β-Blockers, anthelmintics showed relatively lower levels. In the future, additional studies need to be performed to understand the pathway of anthelmintics and their metabolites in the water system.

TU 056
Degradation of antibiotics by photocatalysis on immobilized titanium dioxide
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Antibiotics are micropollutants which release in the aquatic environment has been a subject of concern for several years as they can give rise to two problems: i) they can have a direct effect on bacteria, either those involved in wastewater treatment (activated sludge, biofilms) or those present in natural aquatic environment where they contribute to river self-cleaning, ii) they can promote the development of resistance to antibiotics and the transfer of antibiotic resistance genes between bacterial species, including those pathogenic to man and animal. Most antibiotic are non biodegradable and an advanced physicochemical process should be applied for their elimination. There are several such processes but photocatalysis is often proposed as it can used part of the solar spectrum as an energy source and is reagent free. However most of the works described in literature are based on titanium dioxide slurries. After treatment these nanoparticles should be recover which is not easy. One solution to this problem is photocatalyst immobilization. However immobilized photocatalysts are generally less efficient than suspended photocatalysts as less surface can be activated. Within this framework tests

of photodegradation of two antibiotics often found in the environment: a veterinary drug, tylosin (916 g/mole, pKa = 7.1) and a human drug sulfamethoxazole (253 g/mole, pKa = 5.7). Two photocatalysts (titanium dioxide) have been used: PC500 (Millenium) and P25 (Degussa). Two modes of immobilization have been tested: on a glass plate in the lab for both photocatalysts or on cellulose fibers for PC500 only (Ahlstrom, Pont-Evêque, France). Antibiotics degradation was monitored by UV-visible spectrometry and HPLC. End products (organic carbon and nitrogen species) were also quantified. Degradation was observed, P25 being more efficient than PC500. Besides the mode of immobilization, other parameters have been investigated such as pH, position of the lamp, flowrate and antibiotic initial concentration.

TU 057

Direct and indirect photolysis of human metabolites of antibiotic sulfamethoxazole

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Both direct and indirect photodegradation processes have been reported to be important removal mechanisms of sulfamethoxazole (SMX) in the environment. However, most of SMX enters the environment as human metabolites, yet their environmental fate is not known. In this study, we assessed the direct and indirect photodegradation kinetics at 3 different pHs of 5 human metabolites of SMX, namely, nitro-SMX, nitroso-SMX, acetyl-SMX, hydroxyl-SMX and SMX-glucuronide. Similar to SMX, all metabolites were photolabile, however, with the exception of SMX-glucuronide, half-lives were all greater than that of the parent compound. The protonation state affects absorbance of the compound and degradation rate. Slowest direct photolysis rates were observed at environmentally relevant pH 8.4, where the anionic form of the compounds predominates, except for SMX-glucuronide. The faster degradation of the latter at basic pH was attributed to SMX-glucuronide missing an acidic functionality; therefore, in contrast to SMX and other metabolites, it remains in its neutral and more photoreactive form at environmental pH.
In presence of sensitizers, humic acid and nitrate, which produce respectively reactive $^1O_2$ and/or triplet excited state dissolved organic matter (DOM) and hydroxyl radicals, SMX metabolites degraded faster. The contribution of indirect photolysis to the overall photolysis was found to be greatest for nitro-SMX (70% in presence of 10 mg/L humic acid). Experiments conducted in lake water (1.19 mg-L-1 NO_3^-; 2.43 mg-L^{-1} TOC) showed degradation rates comparable to humic acid experiments. Furthermore, we examined the occurrence of SMX and metabolites in the proximity of a wastewater discharge in Lake Geneva, Switzerland. Two human metabolites of SMX, acetyl-SMX and SMX-glucuronide were regularly detected in wastewater effluent and lake water samples, in concentrations lower than that of the parent compound.

TU 058

Ubiquitous distribution of sulfamethoxazole in tropical Asian and African waters

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Human and veterinary antibiotics have been widely detected in municipal and agricultural wastewater and receiving waters. Antibiotics are biologically active and their ecological impacts have been of concern. Especially, induction of antibiotics resistance is of great concern. Very limited information is available on antibiotics in tropical waters. Infectious disease is more spreading and more percentage of people suffer the disease in tropical area. This is mainly due to climate conditions (hot and wet) favorable for the incubation of vector microorganisms and insufficient sewer and water supply systems. More infectious disease may cause more usage of antibiotics, leading higher concentrations of antibiotics in the tropical waters. In addition to human medicine, large amounts of veterinary antibiotics are expected to be used in tropical countries due to intensive husbandry and aquaculture activities. However, only very limited information is available on the types and abundance of antibiotics in tropical Asian and African countries. Seven sulfonamides, trimethoprim, five macrolides, lincomycin, and three tetracylines were measured in sewage, livestock and aquaculture wastewater, and river and coastal waters in 7 tropical Asian countries. Sum of the concentrations of the target antibiotics were sub to low ppb levels in sewage and heavily sewage-impacted waters. Most abundant antibiotic was sulfamethoxazole (SMX) followed by lincomycin or sulfathiazole. Average concentrations of SMX in sewage or heavily sewage-impacted waters were 11500 ng/L in Kenya (Nairobi; n = 7), 2930 ng/L in Ghana (Accra; n=7), 1750 ng/L in Vietnam (Hani, Ho Chi Minh, CanTho, n=15), 650 ng/L in Philippines (Manila; n=6), 538 ng/L in India (Kolkata; n=4), 282 ng/L in Indonesia (Jakarta, n=10), 76 ng/L in Malaysia (Kuala Lumpur; n=6). These concentrations were comparable or higher than those in Japan, China, Europe, US, and Canada. Predominance of sulfonamides, especially SMX, is unique in these tropical countries. The higher concentrations and dominance of SMX can be ascribed to lower price of the medicine. Concentrations and compositions of livestock and aquaculture wastewater were highly variable. In many cases, sulfamethazine, oxytetracycline, lincomycin, SMX was predominant among the target antibiotics.

TU 060

Environmental fate, occurrence and toxicity of the antiparasitic pharmaceutical toltrazuril

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Toltrazuril is an organohalogenic endoparasitic pharmaceutical used in the acute and prophylactic treatment of livestock such as poultry, piglets, sheep and cattle against coccidiosis. It is metabolized through stepwise sulfooxidation resulting in pharmacologically active metabolites. Hence toltrazuril is excreted as the parent compound and as two active metabolites; toltrazuril sulfoxide and toltrazuril sulfone, mainly via feces. The massive use of other anticoccidial drugs has resulted in increasing resistance of the parasites [1] as a result the use of toltrazuril is expected to be escalating.
Already in 2003, Boxall et al. [2] expressed concern about this compound and considered, it as a high-priority drug with respect to especially environmental and toxicological concerns. However, today still little is known about its environmental occurrence, fate and toxicity in the open literature. In 2008, concerns were raised by Germany during the Marketing Authorization approval of a toltrazuril product [3]. Toltrazuril and its metabolites may present a risk to the environment due to toxic effects on terrestrial plants and due to a risk for ground water contamination caused by manure application on agricultural fields.
Analytical methods utilizing LC-MS/MS have been developed to quantify toltrazuril and metabolites in aqueous matrices using solid-phase extraction (SPE) and in solid matrices using pressurized liquid extraction (PLE). These methods are applied in this work to determine the occurrence of toltrazuril and its metabolites in drainage water, upper ground water and soil from agricultural fields amended with manure containing toltrazuril. Furthermore, the developed methods have been applied to determine the fate of toltrazuril and metabolites in different abiotic processes such as hydrolysis and photolysis. Toxicity was assessed by performing acute growth inhibition tests on Selenastrum Capricornutum (freshwater algae, ISO guideline 14442), cyanobacteria (OECD guideline 201 and acute immobilisation tests Daphnia Magna (freshwater water flea, OECD guideline 202).
1: Kim M-S, Lim J-H, Hwang Y-H, Park B-K, Song I-B and Yun H-I (2010) Vet. Par. 169:51-56.
2: Boxall ABA, Fogg LA, Kay P, Blackwell PA, Pemberton EJ and Croxford A (2003) Tox. Let. 142:207-218
3: EMEA/CVMP/345752/2008

TU 061

The impact of carbamazepine on concentrations of acridine in river systems in Northern Germany

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Carbamazepine (CBZ) is a pharmaceutical product which belongs to the dibenzazepine group. Due to high usage of the substance, concentrations found in the effluent of waste-water treatment plants (WWTP) ranged from 100-6300 ng/L. In river waters, CBZ concentrations from 30-1100 ng/L were found. A toxicological important degradation product of CBZ is acridine (ACR). However, only scant data exist concerning occurrence of ACR in WWTP. Moreover, ACR has not been analyzed in surface waters so far. In line with our very recent measurements of heterocyclic compounds in river systems in Northern Germany, relatively high concentrations of up to 55 ng/L for ACR and for CBZ of up to 320 ng/L were detected. Surprisingly, concentration ratios of ACR to CBZ in different rivers were found to be similar. Based on these results experiments were conducted to explore if concentrations of ACR in river water were influenced by degradation reactions of CBZ.
Monthly water samples were taken between May-November 2011 in a water section of 4.6 km from the River Fuhse. Additionally, water samples from the River Lippe were taken in November in a water section of 7.6 km. Samples were filtrated (glass filter 1.6 µm) and immediately enriched on SPE cartridges (Lichrolut EN, Merck). A fractionation on the SPE-cartridge was performed using different pH-values. Extracts were concentrated to 0.5 ml by using a rotary evaporator and analyzed by LC-MS/MS (Agilent 6430). Recoveries obtained at two concentration levels were 84% for ACR and 70% for CBZ. Photolysis experiments were performed with CBZ for artificial and natural water samples (River Fuhse) using a Xe-light source with a 290 nm optical filter.
Seasonal c_{ACR} values in the River Fuhse range from 20-55 ng/L and c_{CBZ}=120-320 ng/L. Concentrations were a factor of 4-5 higher compared to the larger River Lippe. In order to render concentrations and discharges comparable, the ratio $R=c_{ACR}/c_{CBZ}$ was calculated. Surprisingly, similar values of R=0.13-0.15 were found for both rivers in November, even comparable to values found in the effluent of WWTP. The ratio increases to 0.33 in summer months in the River Fuhse. Besides the presentation of concentration levels and ratios R for both compounds in rivers and WWTP, photolysis experiments and a model are presented on the poster to discuss the impact of degradation reactions of CBZ on concentration levels of ACR in river systems.

TU 064

European environmental risk assessment for Trimethoprim in surface waters

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An environmental risk assessment for the aquatic compartment was developed for the old antibiotic Trimethoprim (CAS 738-70-5). The exposure assessment is based on documented human use figures in western Europe from IMS Health and average removal in wastewater treatment; this predicted environmental concentration (PEC) is compared with measured environmental concentrations (MECs) from Europe. On the effects side, acute and chronic ecotoxicity data from the literature were complemented by additional, new chronic results for fish; based thereon, acute- and chronic-based predicted no effect concentrations (PNECs) were derived. The risk assessment compares surface water PEC and MECs with aquatic PNECs for Trimethoprim.

TU 065

Comprehensive evaluation of the impact of Triclosan in the terrestrial environment

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A comprehensive evaluation of the effects of the antimicrobial personal care product triclosan (TCS) in the terrestrial environment was performed. This evaluation included the effect of TCS on soil microflora respiration and nitrification, impact on terrestrial arthropods, acute and chronic toxicity to earthworm (Eisenia fetida), and effects on emergence and growth terrestrial plant species. Microbial respiration and nitrification were evaluated in soil treated with 0.1-2.0 mg/Kg TCS for 28 days. The acute and chronic toxicity of TCS to earthworms were tested in an artificial soil in accordance with OECD guidelines 207 and 222. Test concentrations for acute and chronic toxicity studies with earthworms ranged from 64-1,026 and 0.3-100 mg/Kg soil (dw) for acute and chronic studies, respectively. The effects of TCS on the predatory mite Hypoaspis aculeifer exposed to 0.3-320 mg/Kg (dw) TCS for 14 days were evaluated in accordance with OECD guideline 226. Phytotoxicity studies evaluating survival, emergence, shoot biomass and length, and normalcy of development in 10 species of terrestrial plants were performed in accordance with OECD guideline 208 using 0.2-1,000 mg/Kg (dw) TCS for an exposure period of the median emergence time plus 14 days. No Observed Effects Concentrations (NOEC) and Lowest Observed Effects Concentrations (LOEC) for soil respiration and soil nitrification were 2 and >2 mg/Kg (dw), respectively. NOEC and LOEC values for acute and chronic toxicity to earthworms were 1,026 and >1,026 mg/Kg (dw) [survival], and 100 and >100 mg/Kg (dw) [survival and reproduction], respectively. NOEC and Lowest Observed Effects Concentrations (LOEC) values for predatory mite survival and reproduction were 10 and 32 mg/Kg (dw) and 3.2 and 10 mg/Kg (dw), respectively. NOEC and LOEC values in terrestrial plants ranged from 75 and 100 mg/Kg (dw) [biomass in lettuce] to 1,000 and >1,000 mg/Kg (dw) [emergence and phytotoxicity in vetch and phytotoxcity in cucumber]. Overall, these studies demonstrate that TCS poses minimal risk in the terrestrial environment which is consistent with Reiss et al. (2002).

TU 066
Risk assessment of pharmaceuticals incidentally discharged to the terrestrial environment
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The Environmental Risk Assessment (ERA) of pharmaceuticals mainly considers the aquatic compartment, since the majority of active pharmaceutical ingredients (APIs) are hydrophilic. However, some APIs with more hydrophobic physical-chemical properties (e.g. Log Koc>4), may partition to sewage sludge during municipal sewage treatment, and following application of sludge to land as fertilizer, there is a potential for chemical residues in soils. With the rapid growth of the human population, water re-use practices are diverse and increasing globally. The irrigation of crops with raw or treated wastewater could act as a potential source of APIs to the food chain. Exposure via this route and its significance is not currently considered within ERA guidelines. The disposal of unused medicines is also of interest, and recent monitoring studies have shown that drugs disposed to landfill can potentially leach out and contaminate local groundwater and surface water.
This poster presentation describes a risk assessment for pharmaceuticals associated with emission potential routes to the terrestrial environment: application of biosolids to agricultural land; wastewater irrigation of agricultural land; unused medicines to landfill. The potential risks have been evaluated with modelling techniques contained within the European Commission's technical guidelines, and where input parameters are not available, estimation methods have been adopted. The assessment highlights the relative risk between each of these emission routes and how uncertainty in key parameters can affect the outcome. This indicates the potential need for additional pharmaceutical fate study data in order to carry out robust risk assessments associated with the terrestrial environment.

TU 067
The ScenAT exposure model: a novel spatial method to inform environmental risk assessments of personal care products in China
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China's economy has grown significantly and so has the demand for home and personal care (HPC) products. The detection of chemicals used in HPC products is increasing in profile as China develop models for use in prediction of environmental concentrations (PEC). Data required to accurately determine PEC's of HPC chemicals include product usage information, physico-chemical properties, environmental behaviour (e.g. persistence, adsorption) and use of environmental information (e.g. receiving water bodies, sewage treatment plant (STP) infrastructure). A GIS-based exposure estimation framework has been developed to predict the fate of HPC chemicals in freshwater ecosystems allowing PECs to be generated at a more spatially resolved level (e.g., 2769 counties in China). Per capita water use values were sourced at the province level and the population connected to STP's were calculated using per capita water use and the STP domestic consumption discharge. Dilution factors were caculated per country using the runoff, contributing area and waste water flow. Predicted environmental concentrations were determined using the fraction removed in STP's or in river, the domestic water use per capita, the annual tonnage of ingredient used, the population dilution factors in receiving waters. The highest PEC's can be seen in North and East China. These areas have increased water scarcity and contain larger densely populated cities like Beijing and Shanghai that will contribute to the increased concentrations. Model validation is difficult due to the rarity of long term measurements of down the drain chemical concentration. Therefore a qualative assessment was perfromed to determine if spatial patterns of water quality were consistent using data published by the Ministry of Environemtnal Protection. The results show the PECs are qualitatively consistent with other spatial assessments of water quality. The model fits neatly between a unit world approach (e.g. EUSES, RAIDAR and USEtox), which are quick and easy to run and catchment models (e.g. GREAT-ER and PhATE) which give a better approximation of real world but are complex and data heavy.

TU 068
A concordance assessment of Predicted-No-Effect-Concentration (PNEC) aquatic toxicity data for pharmaceuticals
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Environmental safety assessments are required for market approval of new Active Pharmaceutical Ingredients (APIs) (in the EU and to a lesser extent in the US) , however, such assessments may be lacking for older drugs. To determine whether available environmental toxicity data can be used to make PNEC predictions for APIs for which effect data are lacking, we analyzed publically available data for 197 APIs. The APIs included in the Stockholm County Council booklet, 'Environmentally Classified Pharmaceuticals' - January 2011 Edition were used as a representative sample of the universe of APIs for the purposes of the analysis. For each of the APIs indicated in the booklet as having a PNEC generated (195), and an additional two, the supporting data were extracted from the Swedish National Formulary website (www.FASS.se) and parsed to facilitate statistical treatment. Following an outlier analysis, the dataset was used to evaluate statistical correlations between the PNEC and trophic level (algae, fish, invertebrate), benchmark type (EC50/NOEC), assessment factor, and Anatomical Therapeutic Chemical (ATC) Classification System group. Based on the findings of the statistical analyses, we consider two potential approaches for generating PNECs for APIs for which no effect data are available, discuss their strengths and weaknesses and future development needs.

TU 069
Environmental risk assessment for the polycyclic musk AHTN and HHCB in the Molgora river (Lombardia Region, Italy)
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Polycyclic synthetic musk (PCMs) compounds are used as fragrances in a wide array of personal care products. The two most important PCM compounds are galaxolide (HHCB) and tonalide (AHTN), used respectively at amounts of 1473 and 385 t in Europe in 2000. In Europe such substances are classified as High Volume Product Chemicals. The removal of both substances during municipal sewage treatment process has been estimated as being approximately 40-60% of the initial amount. For these reasons, it is not surprising to find AHTN and HHCB as contaminants of the aquatic environments. Indeed, the concentrations detected in environmental samples range from ng/L to µg/L in surface waters and effluents. The fact that both compounds show high values of log Kow and potential for bioaccumulation raised serious concerns on their potential adverse effects on the aquatic ecosystems. Furthermore, it has been suggested that both chemicals can act as chemosensitizers and could therefore have indirect effects by allowing normally excluded toxicants to permeate cells. Literature data, reporting the presence of PCMs in the Italian aquatic systems, are scarce and limited. Very recently, a monitoring survey for HHCB and AHTN started in 2010 along the Molgora river, located in a very densely populated area of Lombardy Region (Italy).Water samples were taken seasonally in several monitoring stations located up and downstream of the three sewage treatments plants (STPs) present along the river. Objective of this study is a spatial and temporal risk analysis for the Molgora aquatic ecosystem by comparing the Predicted No Effect Concentration (PNECwater and PNECsediment), derived for aquatic and sediment dwelling organisms, with the relative measured concentrations of AHTN and HHCB in water and sediments.
The observed concentrations of both chemicals dissolved in water are far below the risk threshold, while HHCB adsorbed to particulate organic matter can pose a risk for aquatic sediment dwelling organism. A temporal and spatial variability in PCM concentrations has been observed and the risk to the aquatic communities varies along the river and through the year. In particular, during the reduced water flow, the risk characterization highlights that the entire river downstream the first STP is at risk for the presence of polycyclic synthetic musks.

TU 070
Does price pressure on medicines result in more pollution?
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Pharmaceutical industries outsource more and more of their manufacturing of active pharmaceutical ingredients (API) to low income countries. We have previously shown that some bulk drug manufacturers in Patancheru, India pollute the surrounding environment with very high concentrations of a variety of APIs, including broad-spectrum antibiotics. Additional examples in other parts of the world suggest that this is not an isolated problem. However, information on by whom, where and under what circumstances the API of a given pharmaceutical product is produced is not publically available. This effectively prevents prescribers and consumers to make environmentally informed decision, and thus reduces incentives for API manufacturers to invest in green production technologies. The Swedish Medical Products Agency has information on where and by whom the API is produced for products approved for the Swedish market, data which can be accessible for research purposes. A smaller, previous study of ours revealed that the factories producing the API for 31% of 242 investigated products sent their waste to a treatment plant in Patancheru with insufficient waste water treatment. This disturbingly high figure can however not be extrapolated to the entire Swedish market since we preselected 9 APIs known to be present in the effluent of the Indian plant. Also, actual sales figures were not accounted for. The aims of the present study are therefore to describe where the APIs in pharmaceutical products, sold in Sweden, are produced and to what extent the price of interchangeable products correlate to the country of origin of the API. We will also investigate if price correlates to corruption and environmental performance indexes in the manufacturing country. Such surrogate measures are used as conclusive information on pollution level is lacking for most

production sites. Furthermore, we will analyze if there are general differences between the country of origin of the APIs in generic and original products. To accomplish this we are analyzing sale statistics (price) and information on the production site for APIs for roughly 60% of all products (7836) that were sold on the Swedish market in 2010. To date, all data has successfully been compiled into a workable database. We believe our analyses will highlight some of the international aspects of the environmental challenges linked to pharmaceutical production.

TU 071

Do pharmaceuticals with evolutionary preserved drug-targets in non-target organisms pose a greater environmental risk?

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Human pharmaceuticals are designed to target specific molecules involved in a particular metabolic or signaling pathway in humans to obtain desirable effects at low doses. Although not all of these drug-targets are present within the diversity of biological life found in nature, some are evolutionary well-preserved. It has therefore been hypothesized that non-target organisms with highly conserved human drug-targets may be at a greater risk to be affected by pharmaceutical residues in the environment. In this study, we tested the hypothesis that pharmaceuticals with well-preserved drug-targets in the cladoceran Daphnia magna have greater effects on gene expression and RNA content. Three different pharmaceuticals were tested: the anti-fungal drug miconazole, the anti-histamine promethazine (both with highly conserved drug-targets in D. magna), and the progestogen levonorgestrel (with a low drug-target homology). For each pharmaceutical, juvenile daphniids were exposed to sublethal concentrations and raised to instar 3. The effects were assayed at the level of (1) gene expression (vitellogenin and cuticle protein mRNA using β-actin as a housekeeping gene), (2) relationship between total RNA and DNA (as a proxy for protein synthesis rate), and (3) body size. The exposure to miconazole resulted in a two- and three-fold decreased gene expression of vitellogenin and cuticle protein genes, respectively, as well as a significant increase in RNA content in relation to DNA. The down-regulated expressions of vitellogenin and cuticle protein genes are indicative of endocrine disruption, whereas the increase in RNA can be a result of enhanced protein synthesis as a response to stress. By contrast, promethazine and levonorgestrel did not alter RNA-DNA relationship. No effects on the body size were observed for either test substances. Thus, our preliminary results indicate that for miconazole, the drug with well-conserved drug-targets, the endocrine and protein synthesis disrupting effects were indeed observed at sublethal concentrations. To fully test the hypothesis, complementary analyses on the other two pharmaceuticals are being conducted.

TU 072

Androgenic activity of levonorgestrel in three-spined stickleback (Gasterosteus aculeatus)

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Synthetic progestins are widely used in contraception and have emerged as widespread contaminants in the aquatic environments. They have been shown to impair reproduction in fish at concentrations in the low ng L-1 range. The mechanisms behind the reproductive toxicity of synthetic progestins are largely unknown. Some synthetic progestins, such as levonorgestrel (LNG), exert androgenic effects in humans and other mammals by binding to the androgen receptor (AR). Studies in fish indicate that AR activation by progestins may partly mediate their reprotoxic effects, and cause female fish to develop male secondary sex characteristics. In order to acquire a quantitative determination of the androgenic potency of progestins in fish, we exposed three-spined stickleback (*Gasterosteus aculeatus*) to LNG. Male sticklebacks synthesize a unique glue-like glycoprotein called spiggin, which is used in nest building. Spiggin is produced in the kidney under the influence of androgen binding to the AR. Spiggin production is absent in females but can be induced by exogenous AR agonists, and serves as the best known biomarker for androgens in fish. Adult stickleback females in duplicate groups of eight were submitted to semi-static aqueous exposure to LNG at 5.5, 40 and 358 ng L-1 for 21 days. The effects of LNG on the mRNA expression of spiggin and vitellogenin, kidney epithelium height and organosomatic indices were studied. Spiggin and vitellogenin transcripts were measured using real-time quantitative PCR. Kidney epithelium height was determined in histological sections. The results show a clear androgenic effect of LNG at concentrations ≥40 ng L-1. Spiggin expression, nephrosomatic index and kidney epithelium height were all significantly increased at concentrations ≥40 ng L-1, and vitellogenin expression was significantly reduced at 358 ng L-1. Our in vivo quantitative data show that LNG acts as a potent androgen in fish, giving support to the contention that androgenic effects of certain progestins may contribute to their reproductive toxicity.

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TU 074

Transcriptome analysis of the brain of the gilthead sea bream (Sparus aurata) after exposure to environmental concentrations of human pharmaceuticals

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Pharmaceuticals are being released into the environment in extremely large quantities on a regular basis. Ingested drugs are eventually excreted from individuals, as well as they are released from manufacturing and disposal of unused or expired drugs ultimately winding up in the effluent of wastewater treatment plants and aquatic environments. In order to assess the long term effects of exposure to environmentally relevant concentrations of representative pharmaceutical compounds we have exposed the gilthead seabream (Sparus aurata) to representative drugs found at ng-µg-L-1 levels in surface- ground and coastal waters. Acetaminophen (APAP), Carbamazepine (CBZ) and Atenolol (AT) were used as model compounds to assess the effects of low level, long term exposure on the expression of the brain transcriptome.

The treatment with the greatest number of differentially expressed genes was CBZ with 467 features, compared with 226 for APAP and 4 for AT. The selected vehicle, DSMO, didn't show any significant expression changes in comparison with the SW control. Out of all these features, only one was common between all the three treatments. This feature was identified by homology search as Sparus aurata cytochrome c oxidase subunit I mRNA. No other feature was common both between APAP and AT nor CBZ and AT. However, there were 130 common features between the APAP and CBZ treatment.
Functional annotation clustering and enrichment analysis of APAP and CBZ candidate genes in DAVID revealed treatment specific activation of different pathways and processes, with energy-related features present in all the applied treatments.

TU 075

Chronic effect of atenolol on physiological indices in rainbow trout (Oncorhynchus mykiss)

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Atenolol is a representative of second generation of the beta-blockers, which is often detected in the surface water, due to its extensive use and relatively high stability. In this study, the negative effects of atenolol were studied on juvenile rainbow trout, Oncorhynchus mykiss, by chronic semi-static bioassay. Fish were exposed to sublethal concentrations of atenolol (1, 10 and 1000 ug/L) for 42 days. Multiple biomarkers were measured, including morphological indices, haematological parameters, antioxidant responses and CYP450 activities. Parameters measured in this study displayed various dependent patterns to atenolol concentrations and exposure time. In short, the multiple responses in fish indicated that atenolol induced physiological stress and could be used as potential biomarkers for monitoring residual atenolol in aquatic environment. Molecular and genetic mechanisms of these physiological responses in fish are not clear and need to be further studied.

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TU 076

Investigating the genotoxicity effects of pharmaceutical photo-transformation products

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All types of urban wastewater discharge and reuse practices, including irrigation of landscape and agricultural areas, groundwater replenishment, discharge into inland surface waters and sea, cause the release of organic xenobiotic substances into the environment. Amongst them, pharmaceuticals, which are widespread-used and dissimilar chemical compounds, specifically designed to interact with living organisms, represent an important concern for humans and the ecosystems. It has already been proved that conventional treatment taking place in urban wastewater treatment plants is incapable of eliminating these substances and as a result pharmaceuticals, their metabolites and/or transformation products are released in the environment. Research has been concentrated mainly on the effects the parent compounds may have; whereas investigation of possible effects caused by metabolites and transformation products is still at its early stage.
This study has been focused on investigating effects of photo-transformation products of ofloxacin, a photolabile second generation fluoroquinolole, widely used and frequent found in the environment. Photo-transformation of ofloxacin was achieved by photolytic and photocatalytic treatment at various times. Nine photo-transformation products and degradation mechanisms based on piperazinyl dealkylation and decarboxylation are proposed. Furthermore, their genotoxicity using the cytokinesis-block micronucleus assay with the hepatocellular liver carcinoma cell line (HepG2) was studied and demostranted that a long irradiation time (greater than 30 min) is required to eliminate possible genotoxic effects.

TU 077

Ecotoxicity of 14 serotoninergic pharmaceuticals in the crustacean Daphnia magna, the microalgae Pseudokirchneriella subcapitata and the marine gastropod Haliotis tuberculata

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The serotonin, also named 5-hydroxytryptamine (5-HT), acts both as a neurotransmitter or as an hormone, depending on its localization. The presented work investigated the ecotoxicological effects of 14 pharmaceuticals having serotoninergic properties, i.e. enhancing the effects mediated by serotonin in the central nervous system. The tested

molecules are mainly antidepressant (fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, clomipramine, amitriptyline, mianserin, milnacipran, duloxetine, venlafaxine) but also antipsychotic drug (clozapine), cough suppressant/halucinogen drug (dextromethorphan) or beta-blocker (propanolol). These molecules have the common property to play directly or indirectly a role on the serotonin transporter and/or serotonin receptors. The 14 molecules were tested for their ecotoxicological effects using chronic and acute bioassays on the microalgae *Pseudokirchneriella subcapitata* and the crustacean *Daphnia magna*. Additionnaly to these conventional bioassays, a novel bioassay was developped using primary cell culture of hemocytes from the marine gastropod abalone (*Haliotis tuberculata*). This assay was used in order to address the question of pharmaceuticals effects in a marine species. The acquired data were used to build quantitative structure activity relationship (QSAR) modeling in order to (1) determine if the chemical properties of the molecules can explain their toxicity, (2) identify the mode of action of the selected pharmaceuticals.

TU 078

Environmental effects of anticholinesterasic therapeutic drugs on a crustacean species, Daphnia magna

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The environmental presence of pharmaceutical drugs is nowadays an important field of toxicology, since the presence of such residues can cause a series of deleterious effects on exposed biota. This study assessed the ecotoxicological effects of two anticholinesterasic therapeutic drugs, neostigmine and pyridostigmine in *Daphnia magna*. This evaluation involved acute (immobilization and feeding behavior) and chronic (growth and reproduction) responses to different concentrations of the two compounds. The pharmacological effects of neostigmine and pyridostigmine derive from their capacity to induce a reversible inhibition of cholinesterase activity, and have thus been used to treat the human disease myasthenia gravis. We observed alterations at a sub-individual parameter directly related to the anticholinesterasic activity. Our study calculated 48 h-EC_{50} values in the immobilization assay of 167.7 µg L^{-1} for neostigmine, and 91.3 µg L^{-1} for pyridostigmine. In terms of feeding behavior, we calculated a 5 h-EC_{50} for filtration rates of 7.1 and 0.2 µg L^{-1} for neostigmine and pyridostigmine, respectively; for the ingestion rates, the calculated 5 h-EC_{50} values were, respectively, 7.5 and 0.2 µg L-1 for neostigmine and pyridostigmine. In the reproduction assay, the most affected parameter was the somatic growth rate (LOECs of 21.0 µg L^{-1} and 2.9 µg L^{-1} for neostigmine and pyridostigmine, respectively), followed by the fecundity (LOECs of 41.9 µg L^{-1} and 11.4 µg L^{-1} for neostigmine and pyridostigmine, respectively). We also determined a 48 h-IC_{50} for cholinesterase activity of 1.7 and 4.5 µg L^{-1} for neostigmine and pyridostigmine, respectively. These results demonstrated that both compounds are extremely toxic for *D. magna* at concentrations in the order of µg L^{-1}. By comparing the here-obtained results with the actual concentrations of pyridostigmine previously reported in the aquatic environment (0.22 µg L^{-1}), it is possible that this compound can significantly alter the feeding behavior of *D. magna*. Hence, the ecological implications of these data are of great importance and must be considered under the risk assessment framework of both pharmaceutical drugs.

TU 079

Acute and chronic tests of Tamoxifen and its metabolites on Daphnia pulex

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Drugs administered in humans are eliminated through the excreta in either intact or metabolized form. As other drugs, chemotherapeutic drug residues were measured in wastewater affluent and effluents. They reach continuously the environment where they have the potential to persist. Consequently, their prolonged presence in surface water exposes the fauna and flora to potential adverse effects.

Tamoxifen is a synthetic non-steroidal anti-estrogenic analog inhibiting competitively estrogen receptors. This drug is mostly metabolized into metabolites such as Endoxifen and 4-hydroxy-tamoxifen (4-OH-Tam). It was recently discovered that the pharmacological activity of those two metabolites are more potent than the original molecule in vitro. As no literature exists on acute and chronic effects of these metabolites on aquatic organisms, Daphnia pulex have been exposed to Tamoxifen and its two metabolites in acute and chronic bioassays. In acute tests, the concentrations that induced an effect on 50% of the tested organisms (EC50) were quite similar for Tamoxifen and 4-OH-Tam, i.e. 0.52 and 0.60 ug/ml respectively. Even if endoxifen showed higher EC50 (1.09 ug/ml) signs of distress were observed on daphnids exposed to low concentrations of this molecule (0.79 ug/ml) such as lethargy compared to the control. On-going chronic tests will determine whether the side effects reported during acute tests are observed on Daphnia and their offspring exposed to low drug concentrations. In these tests, Daphnids (<24h) are placed individually in increasing concentrations (between acute NOEC/100 and acute NOEC) until juvenile instar is reached. The number of neonates produced by each mother, as well as any kind of other stress signs, is recorded for 21 days, and the viability of the neonates is also evaluated. Such tests should give a better assessment than acute tests of the potential ecotoxicological impact of Tamoxifen and its metabolites on aquatic ecosystems.

TU 080

Calmodulin inhibition as a mode-of-action of antifungal imidazole pharmaceuticals in non-target organisms: implications for mixture toxicity assessment

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Although mixture toxicity is not addressed in current regulation of pharmaceuticals chemicals, several theoretical models have been developed and applied to predict mixture toxicity in environmental research. The Concentration addition (CA) model describes the mixture toxicity of components with similar mode of action (MOA).
Imidazoles are primarily known as antifungal substances interfering with synthesis of ergosterol in fungi. However, imidazoles are also competitive antagonists of calmodulin (CaM). CaM is a calcium binding protein expressed in all eukaryotic cells, where it participates in many signaling pathways (e.g. nitric oxide signaling), thereby affecting many different cellular functions, particularly neurosecretory activity. Since arthropods are unable to synthesize sterols relying on dietary sources, the sterol synthesis inhibition is not likely a primary mode of action. Here, we hypothesized that exposure to imidazoles disrupt CaM-dependent nitric oxide synthesis in microcrustaceans. Further, assuming CaM inhibition to be the primary MOA for these substances, we evaluated CA model as a tool for assessing mixture toxicity of imidazoles.
We tested our hypothesis by assessing single and combined effects of four imidazoles (econazole, miconazole, clotrimazole and ketoconazole) in adult specimens of the crustacean *Nitocra spinipes* (Copepoda, Harpacticoida). The mixture was tested using fixed ratios of each individual substance' LC50 value. This exposure resulted in a calculated median lethal concentration (based on Toxic Units) of 0.86 (95% CI: lower 0.74; upper 1.01), which shows that the mixture displays concentration additive toxicity, in agreement with the CA model. In parallel, levels of CaM, nitric oxide and nitric oxide synthase were monitored to support the hypothesized MOA of imidazoles for non-target organisms and to justify the application of CA models for assessment of imidazole mixture toxicity.

TU 081

Behavioural and physiological responses to pharmaceutical exposure in Gammarus spp. and Fucus vesiculosus

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Gammarus spp. and Fucus vesiculosus are two foundation species of the Baltic Sea. These species inhabit the littoral community and are thereby exposed to substances released in coastal areas. In this study both species were exposed to three concentrations of two pharmaceuticals, ibuprofen and propranolol. Both physiological and behavioural parameters were estimated to examine potential effects in the organisms. Respiration, feeding rate, activity with and without predator cues by a Multispecies Freshwater Biomonitor (MFB[TRADEMARK]) were estimated for Gammarus spp. and gross production to respiration ratio (GP/R) and chlorophyll fluorescence for F. vesiculosus. Ibuprofen did not affect any of the measured parameters of Gammarus spp. significantly and only slightly affected the algae. The strongest effect was related to activity of Gammarus spp measured by the MFB[TRADEMARK], and results showed that propranolol decreased the activity. The addition of predator cues into the exposure water increased the activity in all treatments, but the gammarids could not compensate for the reduced activity caused by the pharmaceutical. The feeding rates of Gammarus spp. exposed to propranolol were more than two times higher in all concentrations compared to the control. Also the alga was more affected by propranolol measured as lowered GP/R-ratio. These data indicate that the effects on both behaviour and physiology of Gammarus spp. in combination with the stress responses in the alga, might cause unexpected indirect and cascade-effects which have implications on the littoral communities.

TU 082

Acute effects of psychiatric drugs on D. magna under insecticide exposure

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The rates of production, release and use of pharmaceuticals and of psychiatric drugs in particular is expected to increase in the next 10 to 50 years for several reasons, and so is the loading into the environment. In recent years, there has been a growing concern about ecotoxicological risk of these psychiatric drugs that interact with the central nervous system. They include antiepilectic drugs, which decrease overall neuronal activity and antidepressants, which inhibit the re-uptake of serotonin. Indeed, these compounds have been detected in many countries in sewage treatment plant effluents, surface waters, seawaters, groundwaters and some drinking waters. However, little is known about effects of the interactions between psychiatric pharmaceuticals and other substances like neurotoxic pesticides. Because neuroactive insecticides like are usually present in aquatic environments and can co-occur with psychiatric pharmaceuticals it is important to assess this possible interaction
In this work we assessed the acute toxicity of the antidepressant Fluoxetine and the antiepileptic carbamazepine (some of the more prescribed psychiatric drugs) on *Daphnia magna* mortality under exposure to carbaryl or fipronil. D. magna was exposed to binary mixtures of each pharmaceutical and an each insecticide in full factorial designs. To address mixture effects, the observed mortality was then compared to the expected effects of mixtures calculated from effects of single compound exposures, based on existing conceptual models for the evaluation of contaminant mixture exposures: concentration addition (CA) and independent action (IA).
Although the risk for acute toxic effects of psychiatric drugs is unlikely, our results contribute with ecotoxicological data for risk assessment of psychiatric pharmaceuticals in the aquatic environment and are discussed in terms of possible ecological effects of environmental relevant concentrations of these substances on wildlife. We advocate that sub-lethal toxicity of these psychiatric drugs should be assessed under relevant exposure scenarios such as co-occurrence with neurotoxic pesticides.

TU 083

Assessing the environmental hazard of mixtures of pharmaceuticals: combined acute toxicity of fluoxetine and propanolol to the crustacean Daphnia magna

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The aim of the present work is to evaluate the acute toxic response of the crustacean *Daphnia magna* exposed to individual pharmaceuticals and mixtures. We tested fluoxetine, a selective serotonin reuptake inhibitor widely prescribed as antidepressant, and propranolol, a non selective β-adrenergic receptor-blocking agent used to treat hypertension. Acute immobilization tests were performed according to OECD 202 and ISO 6341 guidelines. Less than 24 h old daphnids were exposed for 48 h. Six replicate vessels with five individuals per vessel were tested at each treatment level. Single chemicals were first tested separately; estimated EC50 were 7.0 mg/L for propranolol and 7.8 mg/L for fluoexetine. Toxicity of binary mixtures was then assessed using a fixed ratio experimental design. Five concentrations (from 0.5 to 2 total toxic units) and 5 percentages of each substance in the mixture (0, 25, 50, 75 and 100%) were tested. The MixTox model was applied to analyze the experimental results. This tool evaluates if and how observed data deviates from the CA (Concentration Addition) or IA (independent Action) models, and tests if significantly better descriptions of the observed data can be achieved using a set of deviation functions. These functions allow a differentiation between synergism and antagonism, along with deviations based on the dose-level and chemical ratio dependency. The conceptual model of Concentration Addition was adopted in this study, as we assumed that the mixture effect mirrors the sum of the single substances for compounds having similar mode of action. This analysis showed a significant deviation from the CA model that indicated antagonism. Concentrations tested were much higher than those detected in the environment; however these results are to be considered as a first step in an ongoing project aimed at assessing chronic ecological effects of mixtures of pharmaceuticals.

TU 084

Assessing health status of ruditapes philippinarum exposed to caffeine, carbamazepine, ibuprofen and novobiocin using the neutral red retention assay

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Despite the fact that pharmaceuticals have been detected in the environment at the µg L-1 to ng L-1 range, it has been demonstrated they can cause adverse effects at environmentally relevant concentrations. To detect possible adverse effects of a contaminant in the environment, standardized short-term, sensitive and low-cost methods are usually applied to estimate chronic toxicity against organisms. Lysosomal membrane stability (LMS), has been successfully applied as a screening tool to determine the health status of a wide range of organisms. The feeding style and habitat of the manila clam (Ruditapes philippinarum) make this species vulnerable to organic contaminants bound to water and it has been widely used in ecotoxicological studies. LMS was evaluated in clams' haemolymph by the neutral red retention assay (NRRA). Clams were exposed in the laboratory to concentrations of caffeine (psychoactive stimulant), carbamazepine (anticonvulsant and mood stabilizing), ibuprofen (non-steroidal anti-inflammatory) and novobiocin (antibiotic) during 35 days in a semi-static 48 h renewal assay. Filtered sea water was spiked every 2 days with caffeine (0.1, 5, 15, 50 µg L-1), ibuprofen (0.1, 5, 10, 50 µg L-1), carbamazepine and novobiocin (0.1, 1, 10, 50 µg L-1). Stock solutions of pharmaceuticals were prepared in DMSO (0.001%) to ensure solubility. Test with this solvent was undertaken to ensure no solvent effect. Results showed that neutral red retention time (NRRT) measured at the end of the bioassay was significantly ($p < 0.05$) reduced (50 %) ($p < 0.05$) when exposed to environmental concentrations of pharmaceuticals (caffeine = 15 µg L-1; ibuprofen = 5 µg L-1; carbamazepine =1 µg L-1 and novobiocin = 10 µg L-1). Results showed dose-dependent effect of pharmaceuticals ($p< 0.05$). Clams analyzed after exposure to 10, 15 and 50 µg L-1 were considered to present a diminished health status (NRTT < 45 min) ($p < 0.05$). LMS appears to be a sensitive tool that enables evaluation of the health status of clams after exposure to concentrations of selected pharmaceuticals under laboratory conditions and could thus be used as a biomarker for pharmaceutical contamination in aquatic environments.

TU 086

Cyto-genotoxic effects induced by cocaine on the freshwater bivalve Dreissena polymorpha

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The increase in global consumption of illicit drugs has caused both social and medical problems, but also the onset of a potential new environmental hazard. After human consumption, in fact, drugs of abuse enter the aquatic system posing a potential risk for aquatic biocoenosis. Even if many studies have showed the presence of both drugs and their metabolites in freshwater in the high ng/L to low µg/L range worldwide, at present any information on their potentially harmful effects on non-target organisms is available. The aim of the present study was to investigate the cyto-genotoxic effects induced by the cocaine, one of the most used illicit drug in Western Countries, as well as, consequently, one of the most found in the aquatic environment. Our goal was reached through the application of a biomarker battery on a classical freshwater biological model, the zebra mussel Dreissena polymorpha. The raise of genotoxic effects was investigated by the Single Cell Gel Electrophoresis (SCGE) assay, which evaluated primary DNA lesions, and by the DNA diffusion assay and the micronucleus test (MN test), which investigated fixed genetic damage. The Neutral Red Retention Assay (NRRA), by evaluating the lysosome membrane stability, was used to assess cocaine cytotoxicity. 96 h exposures to three increasing nominal concentrations of cocaine (40 ng/L, 200 ng/L and 10 µg/L), comparable to those currently measured in environment, were performed under semi-static conditions. Our results highlighted that cocaine exposure induced significant ($p<0.05$) increases of both primary and fixed DNA damage, above all at the highest administered concentrations. In addition, since cocaine significantly ($p<0.05$) decreased the stability of lysosome membranes, our data also highlighted its cytotoxicity and the possible implications of oxidative stress for the observed genotoxic effects.

TU 087

A comparison of cyto-genotoxic effects induced by some pharmaceutical and personal care products (PPCPs) on the freshwater bivalve Dreissena polymorpha

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Pharmaceutical and personal care products (PPCPs) are considered new environmental pollutants, since hundreds of these molecules are commonly revealed in the aquatic environment in the high ng/L to low µg/L range worldwide. Among them, the occurrence of the antibacterial triclosan (TCS), the antibiotic trimethoprim (TMP) and the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen (IBU), diclofenac (DCF) and paracetamol (PCM) is well documented both in surface and sewage waters. Notwithstanding, at present, few studies have evaluated their potential toxicity towards non-target organisms. In order to enlarge this topic, sub-lethal effects induced by these therapeutics were investigated by using a multi-biomarker battery applied to the freshwater bivalve Dreissena polymorpha. According to a semi-static in vivo approach, zebra mussels were exposed for 96 h to an environmentally relevant concentration (1 nM), similar for each drug, corresponding to the level measured in surface waters. This choice allowed an easier comparison among their toxicity and gave useful information on their current hazard towards bivalves. The induction of genotoxic effects was investigated on mussel hemocytes by three well-known biomarkers: the Single Cell Gel Electrophoresis (SCGE) assay evaluated primary DNA lesions, while the DNA diffusion assay and the micronucleus test (MN test) investigated the onset of fixed genetic damage. Besides, the Neutral Red Retention Assay (NRRA), by evaluating the lysosome membrane stability of hemocytes, was used to assess the cytotoxic potential of drugs. The biomarker results pointed out that a low environmental concentration of TMP, IBU, DCF and PCM has a slight cyto-genotoxic potential on zebra mussel haemocytes. On opposite, 96 h exposure to 1 nM of TCS was able to significantly increase both primary and fixed genetic damage, as well as to induce significant ($p<0.01$) destabilization in lysosome membranes, suggesting an intense stress in treated bivalves. Even if our data highlighted that, at present, only TCS could represent a serious hazard to bivalves, considering the growing trend of PPCPs use and the time-/concentration-dependencies found for each biomarker after the exposure to selected PPCPs, in-depth studies are an absolute necessity to clarify their true ecological hazard for the aquatic biocoenosis.

TU 088

Toxicity of the antibiotic clarithromycin to green algae (Chlorophyta) and blue-green algae (Cyanobacteria), a comparison of species sensitivity

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Antibiotics are suspicious environmental contaminants as they are biologically active substances. Drugs, which have specific modes of action, may probably cause adverse effects on terrestrial and aquatic ecosystems. Up to now, only a few eco-toxicological studies for the assessment of the environmental risk of antibiotics and their metabolites are available. In Germany, the human macrolid antibiotic clarithromycin is widespread present in surface waters and has been found in concentrations up to 0.3 µg/L. In some studies eco-toxicological effects of clarithromycin on aquatic organisms are published. Unfortunately, most of these findings are not valid according the "Technical guidance document on risk assessment (TGD EU-Commission)", because of missing standardized test methods, missing analytical measurements of the exposed concentrations or else disregarding problems in water solubility and sorption effects. In the present study, adverse effects of clarithromycin on the green algae Desmodesmus subspicatus (Chlorophyta) and the blue-green algae Anabaena flos-aquae (Cyanobacteria) were investigated in compliance with the TGD. As Desmodesmus subspicatus is an established species in toxicity testing on primary producers, Cyanobacteria, a phylum of bacteria, are more and more brought into focus of risk assessors because there is some evidence that they are more susceptible organisms to antibiotics than green algae.

TU 089

Assessment of ecotoxicological effects of environmentally relevant pharmaceuticals and personal care products (PPCPs) using battery of bioassays

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In recent years a group of diverse bioactive chemicals covering pharmaceuticals and active ingredients in personal care products (PPCPs) has raised concern of public and regulatory agencies. PPCPs are used in large amounts throughout the world and are continually introduced to surface waters, mostly by untreated and treated waste waters. PPCPs, some of which have been linked to ecological impacts at trace concentrations, have been detected in various surface and ground waters. Pharmaceuticals are produced as chemicals with specific biological activity for target organisms already in low concentrations. Although they are present in the environment in concentrations of ng/l, pharmaceuticals can elicit biological effects and pose a risk for nontarget organisms. Based on frequent consumption, frequency of detection in the environment and biological activity, a set of priority chemicals has been selected from a broad spectrum of PPCPs (e. g. diclofenac, paracetamol, ciprofloxacin, ibuprofen, methyl paraben, triclosan, tonalide, caffeine, 4 methylbenzilidene camphor). Battery of ecotoxicological bioassays with producers, consumers and destruents (alga growth inhibition test, *Daphnia magna* immobilization test, Microtox) has been used to assess acute toxicity of representatives of several groups of pharmaceuticals and personal care products. In addition to conventional acute ecotoxicological tests, selected chemicals have been tested for specific effects mediated by estrogen and/or androgen receptors. Cytotoxicity and specific activities have been determined by *in vitro* reporter-gene bioassays with mammalian cell lines and recombinant yeast strain. The results show differences among ecotoxicological effects of selected pharmaceuticals and personal care products on the representatives of different trophic levels also depending on their structure, mode of

action and physical-chemical properties. Next to this, endocrine-disruptive activities have been observed for a few of the tested PPCPs. The obtained data indicate which of these environmentally relevant polluting PPCPs pose the greatest risk for aquatic environment.

TU 090

Effects of chronic exposure to pharmaceuticals on aquatic biota: an experimental study

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Purifying techniques at sewage treatment plants are constantly being improved to remove more and more compounds from effluent water but despite this a lot of pharmaceuticals still manage to pass the plants in large quantities. This constant flux of chemically still active compounds into the environment has led to concerns previously because although they are designed to work for humans these compounds can also provoke unexpected effects on aquatic organisms. Although acute effect studies indeed prove that effects on aquatic non-target organisms can occur this usually happens at much higher concentrations than actually monitored in the environment. However, less information on chronic effects is available. From a previous monitoring it became apparent that STP effluent contained relatively high concentrations of metformine, metoprolol, solatol, valsartan, irbestartan, hydrochlorothiazide, diclofenac, carbamazepine, atenolol, and guanylurea. Consequently, it was decided to test these compounds in realistic levels (MIX) next to a control and raw effluent. This poster will present the results of an indoor microcosm study testing the potential impact of pharmaceuticals on aquatic biota in a chronic exposure set-up. Indoor microcosms comprising a simple water-sediment system were seeded with the worm Lumbriculus variegatus, the isopod Asellus aquaticus, the snail Physella sp., and the cladoceran Daphnia magna. Test medium was refreshed every two weeks and test organism abundances were monitored for approximately 8 weeks in all treatments.

TU 091

Toxicity of Ciprofloxacin and Sulfamethoxazole on marine biofilm communities

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Pharmaceuticals are widely used compounds and many of them will eventually end up in the aquatic environment where they might affect various organisms. Two commonly used antibiotics that have been shown to occur in the aquatic environment are Sulfamethoxazole and Ciprofloxacin. As they are used to efficiently treat bacterial infections there is a great risk that they affect environmental microorganism similar to their target organisms if they end up in the environment. Therefore, the concentration-response relationship of these two substances was investigated on natural marine biofilm communities (periphyton) mainly composed by microalgae and bacteria.
Glass discs were submerged in the sea outside Fiskebäckskil on the Swedish west coast and the biofilms colonized the substrate during a week. Thereafter the biofilms were exposed to the two antibiotics (5nmol/L - 9µmol/L) in the lab using the semi static SWIFT periphyton test during 72 hours.
Toxicant-induced effects on the communities were analyzed for both bacteria and algae. Pigments were extracted from the algal part of the communities and the composition was analyzed using HPLC. The pigment composition was used as a way to estimate structural changes in the community. Effects on the periphytic bacteria were measured using the so called Biolog Ecoplates® methodology. The communities ability to utilize different carbon sources is used as a measure on community function.

TU 092

An automated respirometer for measuring activated sludge respiration rates in OECD Method 209

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The activated sludge respiration inhibition test (OECD Procedure 209) is used to assess the inhibitory effects of a substance on the respiration of the heterotrophic population of sewage microorganisms found in the activated sludge sewage treatment process. The original OECD guideline for the test was first issued in 1984 and remained unchanged until July 2010 when it was reissued following an extensive update to improve the statistical basis of the test design. Changes introduced in the new guideline included an increase in the level of replication of test and control mixtures to allow the determination of the No Observed Effect Concentration (NOEC) of a substance, a requirement of Guideline EMEA/CHMP/SWP/4447/00 for the risk assessment of human pharmaceuticals, together with methods for assessing inhibition of nitrification.
Whereas the original OECD 309 could be conducted by one operator with relatively simple equipment, the new requirements for replication call for more complex equipment and higher levels of effort to set up the study. To satisfy these requirements we have introduced and validated a computer-controlled respirometer system comprising 30 incubation chambers each containing an oxygen probe, aeration and headspace nitrogen purge systems and a stirrer to provide mixing and flow of media across the probe membrane. Each probe is connected to an automated, computer controlled data capture system, which records the oxygen consumption by samples of activated sludge in each incubation chamber after aeration has been terminated and the headspace purged with nitrogen.
We describe in detail our experience with the operation of this sensitive equipment and the results that we have obtained with the reference substance 3-5 dichlorophenol and other chemicals tested. We have identified a step-wise approach based on dose-response for the determination of the effects of a substance on activated sludge and recommend that a ring test is conducted to compare the performance of the method and refine the test guideline.

TU 093

Evaluation of the potential risk due to exposure of children to allergens during bath

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The use of personal care products is a common practice in developed countries. This brings as consequence a frequent exposition to chemicals likely to be damaging for human health, particularly in the case of babies and children. Fragrances are a group of chemicals incorporated in most cosmetic and other personal care products including baby care ones. The Scientific Committee on Consumer Products (SCCP), has identified 26 of these ingredients as likely to cause contact allergies. Among products for baby care, those intended for the bath such as shampoos, bubble baths, shower gels, and soaps, contain detergents that can break down the natural barrier of the skin, allowing other irritants and allergens to penetrate.
Based on analytical data previously published providing concentrations of 26 fragrance suspected allergens in bathwater samples, the aim of this work was to estimate the potential risk due to the exposure of children under 3 to these chemicals during bath. With this purpose, the Environmental Risk Assessment (ERA) methodology of the US EPA was applied and three routes of exposition were considered, namely dermal, oral and inhalation. For the compounds under analysis, reference doses were not available in scientific databases to characterize the risk. Hence, RfDs were estimated on the basis of toxicological information such as the NOAEL (Non-Observed-Adverse-Effect Level). The required data could only be completed for 8 of the 26 allergens: pinene, benzyl benzoate, cinnamaldehyde, citral, limonene, hexylcinnamaldehyde, hydroxycitronellal and linalool.
Hazard quotients for each compound and route were calculate and then aggregated to derive hazard indexes by route differenciating 3 age groups: 0-1 years, 1-2 years and 2-3 years. In all cases, HIr for the inhalation route largely exceeded the limit (1) under which adverse effects for human health are not expected, representing the most significant contribution to the total hazard index. The HQ for pinene and limonene contributed in around 99% to the total HIr for inhalation. The HIr for dermal and oral routes were always under 1, being almost negiglible for ingestion and not much significant for skin contact (the larger value was 0.1 achieved for the age group 0-1 years).
As a conclusion, an exhaustive risk assessment is necessary to determine the maximum allowable concentrations in water of personal care products to preserve children's health.

TU 094

Comparative biomarkers responses of zebrafish life stages exposed to oxytetracycline and amoxicillin

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Oxytetracycline (OTC) and amoxicillin (AMX) are antibiotics that are extensively used worldwide. Both compounds are applied in fish and shrimp farming practices, and in other animal intensive farming systems, as a growth promoter or bacterial disease treatment. Their excessive application has become a threat for both human and environmental health. Considering this scenario, our study aims at assessing sub lethal effects of AMX and OTC in embryos and adults of zebrafish. To achieve these objectives a battery of biomarkers were analysed in adults (liver, head, muscle, gill tissues) and embryos (whole body homogenates) exposed to these chemicals. Representative biomarkers from different metabolic pathways were chosen (catalase (CAT), lactate dehydrogenase (LDH), and glutathione-S-tranferase (GST)). No differences in the mortality rate were observed between embryos and adults for AMX and OTC tested concentrations. Adults exposed to OTC showed inhibition of the oxidative stress enzyme CAT in head tissues (96 h-LOECCAThead = 25 mg/L) and an increase in the detoxification phase II enzyme GST (96 h-LOECGSTmuscle =10 mg/L). OTC exposure also induced the GST activity in embryos at the highest concentrations (96 h-LOECGSTembryos =100 mg/L). LDH in adults showed a range of responses according to the different organs analysed: being inhibited in the head tissues (96 h-LOECLDHhead =50 mg/L) and induced in the muscle (96 h-LOECLDHmuscle =10 mg/L) and liver (96 h-LOECLDHliver =10 mg/L) as well as in embryos (96 h-LOECGSTembryos =150 mg/L). Adults exposed to AMX showed an inhibition of CAT in head and gills (96 h-LOECCAThead =1 mg/L and 96 h-LOECCATgill =25 mg/L). An inhibition pattern was also found in GST head tissues (96 h-LOECGSThead=1 mg/L). However, in the gills, muscle and embryos (96 h-LOECGSTmuscle =1 mg/L; 96 h-LOECGSTgills =10 mg/L and 96 h-LOECGSTembryos= 380 mg/L) an induction pattern was observed in the measured GST activity. No changes in LDH activities were notice for embryos and adults exposed to AMX. Antibiotic seem not provoke alterations in liver xenobiotic metabolism at the dosage and time of exposure tested, as expected. Embryos seem to be much less responsive than adults to AMX and OTC short term exposure. Linkages between the enzymatic effects found and parameters at higher levels of organization are essential for the better understanding the effects that of AMX and OTC may have on organisms and the environment.

TU 095

Gemfibrozil and naproxen biodegradation in a river water ecosystem

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Incomplete removal during wastewater biological treatments is the main source of surface water contamination by pharmaceuticals. Degradation of a chemical in the aquatic

ecosystem depends on a variety of factors, including compound properties and environmental factors and above all the presence of a natural microbial community able to degrade it via metabolic and/or co-metabolic pathways. Although pharmaceutical and therapeutic products are widely found in the natural environment, the ecological effects on receiving ecosystems remain largely unknown.
Naproxen, a nonsteroidal anti-inflammatory drug and gemfibrozil, a fibrate drug used as lipid regulator, have been found in several natural waters. They were also found in influent-effluent from a municipal waste water treatment plant inside the city of Rome in and in the receiving River Tiber. For this reason we studied the degradation of these pharmaceuticals in microcosm studies using natural river water. The biotic and abiotic degradation (in terms of the disappearance of the 50% of the initial concentration) of naproxen and gemfibrozil were evaluated in microbiologically active river water versus sterilized one. Moreover, the degradations of naproxen and gemfibrozil were also evaluated in microcosms simultaneously treated with both pharmaceuticals in order to evaluate if their co-presence could affect their environmental fate and the degradation activity of the microbial community. The overall results show that both pharmaceuticals were biodegraded. Gemfibrozil (DT50 > 70d) was more persistent than naproxen (DT50 = 27 d). In the presence of Naproxen the degradation of Gemfibrozil slowed down and its persistence increased to more than 70%.

TU 096
Determination of emerging substance as human waste indicator in the Danube River samples
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Caffeine, methylxantine derivative, is a pharmacologically active substance which stimulates the central nervous system, increases heartbeat rate, dilates blood vessels and works as a weak diuretic. It is present in pharmaceuticals, foods and beverages and is considered to be one of the most commonly consumed drugs in the world. Caffeine can be used as an indicator of human sewage contamination in surface waters because it has no natural non-human sources. The objectives of this study were to determine caffeine presence in the Danube River samples and to evaluate its stability during two months storage of samples on 4°C. Analysis was performed by solid-phase extraction (SPE) followed by new developed reversed phase high performance liquid chromatography (RP HPLC) method. The chromatography was using a Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, i.d., 5 µm particle size) at 25°C, with a mobile phase of 0.1% THF in water (pH 8) - acetonitrile (85:15, v/v). The flow rate was 1 mL/min, and detection by DAD at 273 nm. Samples were collected in July 2011 on seven different locations of the Danube River on a territory of Novi Sad, Serbia. Caffeine amount ranged 0-84 ng/L on the 1st day and 0-52 ng/L on the 30th day after sample collection. On the 60th day after sampling caffeine was under the limit of detection in all water samples. Presence of caffeine confirmed the existence of human waste in the Danube River. Highest values were obtained in sample collected from location near the water supply source "Ratno ostrvo", Novi Sad. Obtained results showed that caffeine amounts significantly decreased during two months storage of samples on 4°C most likely due to its degradation.
The work was supported by Ministry of Education and Science, Republic of Serbia (III46009) and NATO Science for Peace Project 'Drinking Water Quality Risk Assessment and Prevention in Novi Sad municipality, Serbia' (ESP.EAP.SFP 984087).

TU 097
The effects of ibuprofen exposure on fathead minnows (Pimephales promelas)
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Occurrence of the non-steroidal anti-inflammatory drug, ibuprofen, has been reported in aquatic waters in the UK at concentrations ranging between 3-27 μgL^{-1}. Ibuprofen is a widely used prescription and over-the-counter medicine, treating pain, inflammation and fever by reducing the level of prostaglandins through non-selective inhibition of the enzyme cyclo-oxygenase (COX). COX exists in two isoforms; the constitutively expressed COX-1 and the inducible COX-2. In order to evaluate whether pharmaceuticals pose a risk to aquatic organisms, we are testing the hypothesis that any potential effects will be related to the Mode-of-Action of the drug and will be seen at plasma levels in non-target organisms similar to human therapeutic levels.
We have identified the COX-1, COX-2a and COX-2b genes in the fathead minnow (Pimephales promelas). To assess the uptake and effect of ibuprofen exposure, fathead minnows were exposed for 96 hours using a flow-through system to 100 and 500 μgL^{-1} ibuprofen. Four fish were sampled at 3, 24, 48 and 96 hours after exposure. Gills, gonads, gut, heart, liver, muscle and brain were collected to determine the effects (if any) on COX gene expression. RNA isolated from tissues was reverse transcribed to cDNA and amplified by qPCR using specific primers to the COX genes and reference genes (18S and β-actin). Initial results indicate changes in COX gene expression following ibuprofen exposure.
Plasma and water samples were also collected to determine ibuprofen concentrations in the fish and the exposure water, using mass spectrometry. This confirmed that fish were exposed to ibuprofen concentrations close to nominal (105 ± 2.6 and 502 ± 49 μgL^{-1}, respectively). Analysis of the plasma revealed uptake of ibuprofen to be up to 9-fold (100 μgL^{-1}) and 360-fold (500 μgL^{-1}) higher than the nominal concentrations. Variation in ibuprofen uptake was noted between individual fish in the high concentration group (range 120-700 fold), and current work is focussed on relating this to changes in COX gene expression.

EM02P2 - Fate and exposure modelling

TU 099
Large-scale spatial-temporal modeling of historical pesticides applications
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With increasingly more detailed data and faster computers, it becomes easier to model pesticide mass loadings into aquatic systems at a high-resolution with temporal variability for large geographical areas. A large-scale co-occurrence assessment was conducted to quantify spatial and temporal mass loadings of pesticides into tributaries to the Sacramento River, San Joaquin River, and Bay-Delta estuary California. The aim was to guide future risk assessments for sensitive and endangered species. As part of this project, ten years of daily mass loadings were simulated for 40 chemicals in a 164,000 km2 area of California's Central Valley. Environmental fate and transport models used for the analysis included the Pesticide Root Zone Model (PRZM), modified to simulate pesticide losses in irrigation tail water, and the rice water quality model (RICEWQ). Model inputs included historical pesticide use data from the California Department of Pesticide Regulation's Pesticide Use and Registration (PUR) database, daily weather data from 19 stations in the California Irrigation Management Information System, detailed soils information (SSURGO) from the NRCS, and high resolution land use data from the Farmland Mapping & Monitoring Program (FMMP). Pesticide application sites represented in the simulations included fruit, vegetable, grain, nuts, rice, landscape maintenance, and structural applications. Approximately 9,115,000 pesticide applications were represented in the simulations, accounting for a total applied mass of 98,279,000lbs of active ingredient for the 40 chemicals. Approximately 14.2% of the applied amount was predicted to reach surface waters via runoff, erosion, drift, and discharge. Runoff from agriculture accounted for over 86% of the mass losses loadings. Erosion and drift from agricultural applications accounted for approximately 5.0% and 4.4% respectively of mass loadings. Another 4.3% was predicted to discharge and run off from rice paddies. Urban runoff accounted for less than 1 percent.

TU 100
Modelling the seasonal cycle of POPs in soil, vegetation, and cow milk from a high altitude pasture in the Italian Alps
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Pollution in soil, vegetation, fodder, milk and feces from diary cows that graze on a high altitude plateau in the Italian Alps has been measured during the pasture season. We followed the seasonal cycle of some obsolete pesticides (DDT and its isomers and metabolites, HCH and its isomers, HCB) of some congeners of polychlorinated biphenyls (PCB) and some congeners of brominated flame retardants (PBDE) during several pasture seasons that, at those altitude, last typically from June to September. Soil act as the main repository compartment with a high dynamic role because consistent concentration changes were observed over relatively short periods (few months). Typically POPs re-volatilization process from soils occurs just after the snow melting in May/June; the PCBs emission was quantified in terms of several tens of µg/m2 depending from the topographical features. During this period, soil acts as a secondary source of contamination to the pasture, which shows a concentration peak in July. Vegetation confirms itself as an efficient scavenging medium for intercepting POPs fluxes from the air and from the soil during emission periods, and its role was quantitatively evaluated. We also found some evidences that air should be considered as a two layer compartment, dividing the part that is under the vegetation cover and the upper part that is interested by turbulent air fluxes made by winds. The lower layer, when soil is covered by herbaceous vegetation, could be very thin, but nevertheless seems to be extremely important in determining input and output fluxes between soil and vegetation. Vegetation is an efficient pathways for transferring POPs into terrestrial food chains. By monitoring intake and output of pollutants we were able to develop a multicompartimental 'cow model' describing the main fluxes of POPs between the pasture environment. We observed a transfer of pollutants between soils and herbaceous vegetation (that act as a temporary filter) and from vegetation to milk and feces, with fast transfer rates. We also observed differences in PBDE fingerprint between inputs and outputs, pointing out differential absorption and/or metabolism of these compounds in cows.

TU 101
Fate and behavior of pesticide in US soil: use of kinetic modeling to assess environmental relevance of metabolites
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In the European Regulatory context of inclusion of active substances in the positive list of Regulation (EC) N° 1107/2009, authorizing their use as pesticides, studies conducted in US are usually not recommended because of significant differences in experimental design, soil specificities and objectives when compared to OECD guidelines. However when a US soil metabolism study (conducted according to US-EPA guideline i.e. OPTTS 835.4100 using US soils) was conducted and submitted as part of the EU dossier, Regulatory authorities may ask for clarifications on soil metabolites, even formed under the specific conditions of this study type.

The objective of this work is to present a case study where apparent conflicting results were obtained between US-type soil metabolism and EU-type soil metabolism studies: In the US study, additionally to one major metabolite M, two metabolites (m1 and m2) were considered to be formed directly from the parent in the metabolic pathway proposed by the laboratory in amounts continuously increasing up to around 7% of the applied radioactivity until the end of the one year incubation. These results were contradictory to the EU type studies where only M was identified.
Kinetics optimization with ModelMaker software and statistical analysis of the data according to the recommendations of the FOCUS Kinetics Workgroup (2006) were performed.
The kinetic evaluation demonstrated that, using a new metabolic pathway, this optimization of kinetic data leads to an acceptable error. Therefore, these soil metabolites were found not to be environmentally relevant according to EU guidance. This justifies the need to perform additional kinetic investigation of the metabolism of pesticides so as to set up properly the degradation pathway.

TU 102
Determination of plant uptake factors for pesticide fate modelling
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The plant uptake factor (PUF) is defined as the concentration of a compound in the solution taken up by the roots divided by its concentration in the soil porewater. Numerical leaching models applied for the environmental exposure assessment of pesticides use the PUF to calculate the amount of a compound taken up by a plant together with the transpiration stream. The PUF is therefore an important parameter for a realistic leaching assessment.
PUF values have been experimentally derived for four substances covering a wide range of polarity (ionic, polar, medium polar, lipophilic) in three different crops (Tomato, Wheat and Maize).
In the hydroponic experimental set-up intact plants were exposed between 8 and 11 days to a nutrient solution (pH 6) spiked with the radiolabelled test compound. Volume uptake and concentration in the nutrient solution were measured regularly over the experimental period. By sealing the test vessel it was assured that the only loss process from the system for water and chemical was plant uptake.
As a result of the study, the PUF was always well above the FOCUS default value of 0.5 (FOCUS, 2009). Generally, a small variation between plant species was observed. The experimental results could be verified with a simple plant uptake model.
The data on PUF show that the use of the FOCUS default of 0.5 is a very conservative approach in terms of leaching assessment. For the test compounds exhibiting ionic to lipophilic properties and examined in three diverse crops representative for cereals, small grains and vegetables all measured PUF values were well above 0.5. It is expected that these high uptake values can be generalized to other compounds within the same range of polarity, as the plant uptake model successfully applied to confirm these values only uses generic compound parameters as input (e.g. lipophilicity).

TU 103
Development of a French national tool for pesticide risk assessment in the context of the water framework directive
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The main objective of the Water Framework Directive (2000/60/EC) is to achieve good ecological status in all surface and groundwater bodies in the EU by 2015. However, it is already clear that this goal cannot be achieved by 2015 for a number of water bodies in France in intensively used agricultural areas. It is therefore important to have powerful exposure assessment tools which are able to i) link the observed contamination of water bodies with agricultural pesticides with the agro-pedo-climatic conditions and pesticide application practices responsible for the contamination, and ii) explore the effect of risk mitigation measures a priori in order to determine the most effective and efficient measures for later implementation in practice.
FOOTWAYS has been charged with the development of a national pesticide exposure and risk assessment tool for France to be applied in the context of the Water Framework Directive. The tool will produce results at two different scales: i) river water bodies and groundwater bodies, for the purpose of risk assessment and global testing of the effects of mitigation measures, and ii) edge-of-field water bodies, for more specific testing of the effect of mitigation measures. The tool will be part of the FOOTWAYS Pro web platform for pesticide risk assessment and management.
While the first phase of the project is focusing on an evaluation of the current situation, in the second phase also scenario simulations exploring the effects of the implementation of mitigation measures will be conducted. Results from the first two phases of the project will be presented, including exposure and risk estimates for the widely used substance glyphosate and its main metabolite AMPA.

TU 104
Pesticide exposure assessment in flowing waters - Approaches to dynamic predicted environmental concentration
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Pesticide risk evaluation for surface waters in the EU is based on the FOCUSsw standard scenarios ditch, stream, and pond, which are characterized by fixed amounts of water for dilution of pesticide input. The representativity of this water bodies for existing waters in real landscapes of the EU was never verified, and there is some doubt that the FOCUSsw standard scenarios reflect the "realistic worst case"appropriate. Given the fact, that the vast majority of water courses are flowing waters and not stagnant ditches, a more realistic pesticide exposure asessment has to respect some basic properties of rivers:
(i) In flowing waters, the hydrodynamic dispersion lowers the peak concentration but spreads the longi¬tudinal extension of an initial substance pulse.
(ii) Pesticide applications on fields along a river stretch occur stochastically, thus a water package moving downstream the water course may be affected by depositions via spray drift, runoff and tile drainage, respectively, more than one time.
(iii) The locations of pesticide input and the sites where ecotoxicological effects may occur are apart from each other.
The pesticide concentration in flowing waters observed at a point of a river stretch fluctuates irregular, depending on the temporal and spatial pattern of the pesticide input upstream. For the registration procedure these concentration oscillations has to be transformed into an operational exposure indicator, comparable to a regulatory acceptable concentration (RAC). For this purpose the GeoRisk project introduced the quantity $PEC_{TWA}(1h)$, the maximum of predicted environmental concentration averaged over a 1 h time period. The concentration pattern over the entire period of applications is scanned with a moving temporal window of 1 h and then averaged to the 1 h mean.
The poster presents results of the $PEC_{TWA}(1h)$ achieved by Monte-Carlo (MC) simulations for generic water courses with tiered combinations of the factors flow velocity, discharge volume, width-depth ratio, percentage of river stretch receiving spray drift deposition, and duration of exposure time. The timing of the spray drift depositions along the water course was randomized for each MC-realisation of the combinations. Additionally the sensitivity of the variables is depicted. The presentation of Trapp et al. demonstrates the application of the PEC-dynamic approach to a real brook in Germany.

TU 105
Plant uptake of eight pesticides / metabolites as a function of log Kow and of pH in a hydroponic test system
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Plant uptake of environmentally relevant chemicals like pesticides is an important process limiting their availability for leaching, run-off and volatilisation. Chemical hydrophobicity (log KOW) and for ionisable compounds the pKa value are the most important properties determining the ratio between a compound's concentration in the plant-root system to that in the pore water adjacent to the roots. The Plant uptake factor (PUF) can be used as input parameter in simulations models (e.g. FOCUS PELMO) to determine the leaching behaviour of the substance more precisely. The PUF was measured in soil-less hydroponic test systems with intact plant root system in an artificial pore water solution (1-L of 0.01 M CaCl2) containing a given concentration of the substances. By determining the volume uptake and concentration of the test item in the solution at different time intervalls PUFs can be calculated. If 14C-labeled compounds are used the distribution of test item in the root plant system can be simply determined by sample combustion followed by liquid scintillation counting (LSC). The test system used in this study are 1-L brown glass vessels in which the plants without soil (pre-grown in soil for 5-6 weeks) are inserted into the test solution and cultivated for 8 days under controlled greenhouse conditions. For each substance, the experiment is conducted with three different plant species (Wheat, Oil seed rape, Tomato) and under three pH conditions using biological buffers (5.5, 6.5 and 7.5). The PUF of 8 test substances, chosen on a relevant range of lipophilicity and pKa value, will be determined on several sampling dates (0, 2, 5 and 8 days after treatment), as well as in the test plants at the end of cultivation. The PUFs determined will be presented crop specific as a function of the chemical hydrophobicity of the substances, and of the pH-level of the test solution.

TU 106
Exposure and risk assessment for pesticide inputs into surface waters via surface runoff, erosion and drainage: developing a new concept for German national pesticide authorization
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The new EU regulation 1107/2009 concerning the placing of plant protection products on the market requires a harmonization of the various national pesticide exposure and risk assessment approaches with each other. As a first step towards harmonization among member states, the German Federal Environment Agency (UBA) launched a project to harmonize the German national exposure and risk assessment procedure for surface waters with the procedure used at the EU level and in various other member

states (FOCUS surface water). Since a direct adoption of FOCUS surface water for the German national authorization procedure is not feasible for a number of reasons, within the project a new national exposure assessment tool for pesticide inputs into surface waters via surface runoff, erosion and drainage will be developed. This tool will i) take into account the full range of agro-pedo-climatic conditions in Germany, ii) facilitate modelling the effect of risk mitigation measures, iii) include a number of higher-tier options and iv) allow for a coupling of predicted exposure time series with standard laboratory or higher-tier effect time series. The project coordinated by Dr. Martin Bach at University Gießen started on 1 December 2011 and will run for 23 months.
The general outline of the project and results of Work Package 1 (Evaluation of the FOCUS surface water modelling concept with respect to representativity and protectiveness of the included scenarios for German conditions in comparison to the current assessment methods in the German national authorization procedure) will be presented.

TU 107
Impact of recent EFSA guidance on soil degradation rates and environmental exposure modelling
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Registration of agrochemicals under the EU Regulation 1107/2009 requires a lot of challenges and numerous guidance documents have been recently issued, thanks to EFSA for the organization of the release of these. Among them, the methodology of the derivation of kinetic parameters linked to the dissipation of chemicals in the environment is probably the most advanced in the Regulatory arena world-wide. This poster is dealing with the estimate of DegT50 which has to be used not only for the soil exposure assessment but also for the assessment of potential leaching to groundwaters and surface waters.
The general procedures for calculating degradation rates are well known but the assumptions made during this process can appreciably affect the results.
Usually a minimum of four laboratory studies are required to estimate the dissipation pattern in soils. If the degradation half-life (DegT50) in top soil at 20°C at pF = 2-2.5 exceeds 60 days additional field dissipation studies need to be conducted.
In the field, the persistence of pesticides is affected by the varying environment, i.e. soil moisture and temperature having an significant effect on the bioactivity of the microflora. Normalisation of field degradation data may need to be performed to obtain reliable degradation parameters and to be used for modelling pruposes.
FOCUS (2005 & 2006 further to FOCUS,1997) developed detailed guidance on estimating degradation rate parameters from laboratory and field studies.
In the past years, the time-step normalisation procedure as described by FOCUS (2006) has become popular in the EU registration. This procedure assumes that the decline in the field can be described well by numerical models that assume first-order degradation kinetics. The procedure implies that the decline curve after normalisation can be used directly to estimate the DegT50matrix of the top soil at 20°C and pF = 2.
However, according to EFSA panel, this decline is expected to show a rapid initial phase in the period when surface loss processes play an important role followed by a slower phase that is dominated by the degradation rate within the soil matrix.
In the proposed poster, we will show for an existing substance how the methodology is being used (according to the new EFSA (2010) guidance) to derive degT50 and we will discuss the impact of this new guidance on the results and on exposure estimation.

TU 108
How reliable is the predicted leaching of pesticides into Groundwater? - Validation of the FOCUS groundwater model PELMO 4 for use in the German national registration process
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In the registration procedure in Germany the risk assessment for the leaching potential of plant protection products into groundwater is based on modelling results using simulated leaching concentrations of the FOCUS Hamburg scenario from the latest version of the FOCUS PELMO model (tier 1, 2). Dependencies of pesticide sorption and degradation data from different soil properties as well as the parameter variability are considered in the endpoint selection for modelling, which is in line with the FOCUS recommendations. The measured leachate concentrations for active substances and/or metabolites from outdoor lysimeter studies are accepted as higher tier endpoints in the risk assessment and overwrite predicted concentrations from simulation runs (tier 3). This is generally justified by comparable soil and climate conditions in the Hamburg scenario and during lysimeter studies and by the presupposition, that experimental analysis lead to more precise predictions of the leaching potential of such compounds under realistic conditions. However, the implementation of the new FOCUS PELMO model in the German authorisation procedure in combination with the adapted selection of modelling endpoints requires a validation of the national tiered approach. In a current research project, predicted and measured leachate concentrations from modelling and lysimeter experiments are compared for several active substances and their metabolites. Preliminary results are presented and lead to discussions about the reliablility of the predicted potential leaching behaviour of pesticides into groundwater in Germany.
Further investigation is planned on the critical points, how the short duration of lysimeter studies in combination with a single application finally affects the prediction of the leaching behaviour. The methodology of inverse modelling with FOCUS PELMO was chosen to derive degradation and sorption values for selected substances and allow a model based extrapolation of the lysimeter results to different conditions, e.g. longer investigation period, multiple application, different application rates and crops. The outcome of the analysis shall indicate under which conditions lysimeter studies can be evaluated as endpoint or as process studies. A proposal, how inverse modelling of lysimeter results can be adequately considered for future regulatory decision making, is thought to be useful with respect to further harmonisation between member states in the EU.

TU 109
The impact of the rainfall criterion in the new EFSA soil persistence guideline on the results of field degradation trials
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In the new EFSA guidance on soil persistence (EFSA, 2010) it is intended to exclude loss processes on the soil surface (photolysis, volatilisation) for the derivation of field half-lives of pesticides. As a pragmatic approach, it is proposed that only sampling points after 10 mm cumulative rainfall should be used to derive DT50. While this rainfall criterion is meant to ensure that processes on the surface are not taken into account for kinetic evaluation of field studies, there is no clear scientific basis for the selected 10 mm used as a rainfall criterion. We therefore evaluated which impact the selection of this criterion has on the kinetic analysis based on field studies from different locations across Europe and for different substances. Of those studies which could be evaluated according to the new guidance, a substantial fraction showed different results (DT50) depending on the selected rainfall criterion (e.g. 5 mm, 10 mm, 15 mm, etc). While in some cases only the resulting DT50 varied according to the applied rainfall criterion, in other cases studies could not be evaluated anymore. We also show that the impact of the rainfall criterion on the resulting DT50 depends on both the study location within Europe and the time of application. These results show that the selection of the rainfall criterion can have a pronounced effect on the results of field studies. Hence, this criterion needs to be chosen with care. Studies on the speed of movement of pesticides in the soil, depending on substance and soil specific properties and rainfall, may help to show if the currently proposed value of 10 mm is reasonable.

TU 110
InversePELMO a specific software to perform inverse modelling simulations with FOCUSPELMO 4
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In the assessment for authorisation of a plant protection product its leaching beha-viour is an important factor to protect the groundwater. In this context the sorption to soil (Parameter: Koc) and the degradation (Parameter: DT50) are processes that must be taken into account. Recently, the new FOCUS groundwater group suggested a third methodology for the input parameter setting (FOCUS 2009). The idea is to analyse outdoor studies, especially lysimeters, using the inverse modelling method that allows the estimation of Koc and DT50 parameters within a single step. For this procedure an optimisation tool, the program PEST (Model Independent Parameter Estimation), has to be combined with a leaching model (here: FOCUSPELMO 4). The aim of inverse modelling simulations is to find those Koc and DT50 values that could describe the outdoor study best by considering all data recorded during the experiments (e.g. rainfall, temperatures, percolate, and substance fluxes). Therefore all data of a lysimeter study are used to vary the input parameters Koc and DT50 until the leaching model shows the same results as the experiment. Generally, 2 steps have to be conducted when performing inverse modelling simulations. Firstly, the hydrology in soil is optimised, followed by the optimisation of pesticide fate. The software InversePELMO is able to provide both programs (the optimisation tool and the simulation model) with the necessary input files in the correct format. InversePELMO has also a built-in module to perform standard statistical tests to check the quality of the optimisation such as the determination of the 'FOCUS error' at which the chi² error passes as described in FOCUS (2006). It was also tested that PEST works under all relevant windows systems (XP, VISTA and 7) in a 32 bit as well as 64 bit version. The results of InversePELMO can be used to make predictions about the most likely behaviour if the lysimeter study had been conducted over a longer time period, translations of the lysimeter results to a different situation with respect to the environmental conditions (e.g. different climate), translations of the lysimeter result to a different situation with respect to the application pattern of the substance (e.g. change of the rate) and the use of optimised parameter settings for a refined standard tier 1 simulation.

TU 111
Multi-dimensional modelling of pesticide placement and fate in the soil-root-plant system
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Many pesticides are soil applied, including residual herbicides, soil insecticides and seed treatments incorporating fungicides and/or insecticides. They are under intense regulatory pressure with regard to water contamination. The pressure of regulation of pesticides within the EU is such that registrants are examining the option of using application strategies such as seed treatments and slow release formulations as a means of reducing environmental impacts due to off-site movement of pesticides. Experimental approaches for demonstrating environmental benefits of formulation are impractical: each active ingredient is sold in many formulations and the cost of a

full-field environmental programme is barely affordable for a new active ingredient let alone for a single formulation. Simulation models are the primary basis for regulating pesticides against EU ground- and surface-water protection standards, but the current models cannot explicitly represent plants or their roots, or the effects of formulations and formulation placement strategies (e.g. slow-release microcapsules and seed treatments). Other models exist that represent individual components of the system in a more satisfactory way.
Current models used for pesticide registration within the EU are 1-dimensional and have a simplified representation of root development and uptake of pesticides. Conversely, plant growth and uptake models can have sophisticated representation of plant processes, but often do not consider pesticide dynamics. An ongoing project has created a conceptual framework for representing local pesticide concentrations in the whole soil-plant system, and will next produce a spatially-explicit simulation model. By relating local concentrations of pesticides to product efficacy, it will be possible to use the model to design better active ingredients, formulations and placement strategies. The new model will also enable the environmental benefit of advanced formulations and placement strategies to be quantified in a cost-effective way for the first time. This presentation will introduce the project to the scientific community, overview results from the first year of work, and discuss anticipated outcomes and the potential benefits to the scientific and regulatory communities.
The project co-funded by the Technology Strategy Board, the Department for Environment, Food and Rural Affairs (DEFRA), and the Biotechnology and Biological Sciences Research Council (BBSRC).

TU 112
Last advances and perspective for a better risk assessment of the tropical use of Protection Plant Products in France
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Risk assessment of water contamination required prior the pesticides registration for French overseas territories is currently conducted using tools and guidance documents established for EU context although the agro-pedoclimatic conditions of these tropical regions would require to use specific tools and methodologies.
The aims of this communication are to (1) clarify how deriving some pesticide's properties appropriate for tropical soils from EU endpoints with the aid of a statistical analysis performed on degradation and adsorption parameters obtained from temperate and tropical soils, (2) summarize the main specificities of agro-pedoclimatic conditions and transport of pesticide in these regions to account in modelling and (3) provide a brief overview of the principles of the model currently under development and the framework planned to adapt it for regulation.
The statistical analysis shows that EU endpoints seem to be provisional acceptable entries for the risk assessment in these tropical regions but should not supersede any experimental evidence supplied with tropical soils. The knowledge about agropedoclimatic conditions and transport of pesticide in these territories underlines that (1) a minimum of four scenarios (2 soils: andosol and nitisol and 2 crops: banana and sugar cane) would be necessary for the risk assessment of the main uses of the French overseas territories and (2) the models to be used for predicting transport of pesticide have to account for preferential flow and specific redistribution of rainfall by crops canopy for banana. For water surface contamination, the module for surface water fate would likely to be initially simplified to a simple static receiving water body of dimensions defined consistently with French overseas territories and protection goals. Finally, the main features of two models - currently under test- representing surface and subsurface flow of water and pesticides in banana crops on tropical volcanic soils are summarized and the schedule of a 2 year research project (2012-2014) to adapt them for regulatory purposes introduced.

TU 113
Simulation of the herbicides flufenacet and metazachlor in a lowland catchment with the model SWAT - A sensitivity analysis
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TU 115
Spatial differentiated impact assessment of chemicals in freshwater ecosystem at multiple scales
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Spatial differentiation is a topic of increasing interest within ecological risk assessment (ERA) and Life Cycle Impact Assessment (LCIA). A key issue to be addressed in the impact assessent methods, models and corresponding impact factors is the level of spatial detail required and uncertainties related to the use of generic impact factors when the exact location of the activities is unknown. For freshwater ecotoxicity evaluations were performed in order to assess the relative influence of scale in calculation of LCIA characterisation factors, comparign them with the result of site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the differences between a site specific calculation and the result of box nonspatial model, such as USEtox .ERA results are calculated following a site-specific ecological risk assessment procedure that integrates in the assessment the spatial distribution of aquatic ecosystem. ERA results at different scale present higher variability: key drivers of variability and relevant differences in addressing spatial differentiation will be presented and discussed. Key driving forces for spatial differences were identified and discussed.

TU 116
Combination of SFA and multi-media fate modeling to assess the consequences of global trade and informal recycling of WEEE in China
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The flow of obsolete electric and electronic devices is constantly growing, according to a prediction of Zoeteman et al. (2010) by 3 to 5 % each year. It is suspected to add up to a volume of 20 to 50 million tons every year (Brigden et al. 2005). A large amount of waste electric and electronic equipment (WEEE) containing a variety of harmful substances such as PBDE and Pb is shipped overseas to China, India and Western Africa for recycling. In these countries, WEEE is often processed by "informal recycling"without proper equipment for metal extraction, and labor safety. Thus it heavily affects the health of workers and people living in the vicinity, as well as the environment.
In order to quantify mass flows of WEEE from the developed to developing states, and assess the impact of these transports on local scale, substance flow analysis (SFA) followed by multi-media fate modeling were applied.
In the frame of the SFA the following information was gained:
a) Global transportation routes and mass flows of WEEE
b) Data on the composition of typical e-wastes exports with regard to the European WEEE-Directive categories
c) Chemical composition of the single categories with a special focus on lead and brominated flame retardants (PBDEs and TBBPA)
d) Estimated emissions of Pb and BFR to the environment as a consequence of different informal recycling techniques.
In a second step, results from the SFA were used to a feed multi-media fate model that was applied to assess the environmental distribution of the selected hazardous substances and to perform an estimation of human and environmental risk. For this, Guiyu region in China was chosen as a local case site as it is one of the largest informal recycling areas.
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TU 117
Modelling the environmental fate and effect of organic bulk emissions from industrial effluents in LCA
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Several fate and effect models have been developed to assess the ecotoxic impact of individual chemical contaminants on aquatic freshwater organisms in Life Cycle Assessment (LCA). These methods suffer when it comes to the impact assessment of complex chemical mixtures, such as industrial effluents, as the list of constituent compounds is rarely known in its entirety, leading to under- or overestimation of ecotoxicological effects. A more holistic and meaningful method of evaluating the environmental impact of industrial effluents in LCA would be to model the fate and effect of comprehensive and commonly measured sum-parameters, such as total organic carbon (TOC). We present our methodology for establishing aquatic (freshwater) ecotoxicity characterisation factors (CF) for waterborne organic bulk emissions from different industries, based on whole effluent assessment (WEA) and the principles of mixture toxicity. We discuss the advantages of the methodology, its complexities, as well as the need for spatial differentiation, and we present some preliminary results.

TU 118
Globally applicable, spatially explicit assessment of non-toxic air pollutants regarding health impacts due to ozone
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1. Introduction
This poster presents intake factors (iF) and characterization factors (CF) regarding human health effects for the pollutants NMVOC, NOx and SO2 accounting for health impacts due to ozone. Research on life cycle impact assessment (LCIA) for transboundary pollutants have been reviewed and it is suggested that spatially differentiated fate modelling is crucial. The present work aims to fill the gap of consistent global modelling by developing an approach to derive globally applicable and spatially explicit values, and by providing a comparison between models with a different degree of spatial resolution.

2. Materials and Methods
Within the ongoing EU-funded project LC-IMPACT (2010-2012) the "Development and application of environmental Life Cycle Impact assessment Methods for imProved sustAinability characterisation of technologies"is pursued. Within the area of "Non-toxic pollutant impacts"the objective is to develop globally applicable, but spatially explicit, LCIA methods and characterization factors.

Within the TM5-FASST modelling framework, the world is divided into 56 regions. Each region serves as a source region and each grid cell (resolution 1°x1°) of the whole world serves as receptor region. Population data and concentration response functions are applied in order to calculate the iF and relevant diseases. Finally, the health impacts are weighted in order to get DALYs per unit of emission, and different external cost estimates, by including and excluding equity weighting.

3. Results
A detailed comparison will be made between iFs derived by the TM5-FASST model and by the EcoSenseWeb integrated assessment tool in order to assess the inherent uncertainties and implicit variability in the estimates. iFs and CFs are calculated for several countries and larger regions. Finally, iFs and CFs suggested in different LCIA methods have been compared with the findings of this work

Acknowledgements
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TU 119
Environmental impacts of thermal emission to freshwater: Spatially explicit fate and effect modelling
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Power production is a crucial sector causing heavy impact on freshwater bodies by releasing enormous amounts of heat in once-through cooling systems. So far only few approaches have addressed impact on aquatic environment related to this issue and there is no study providing fate and effect model for assessing the impacts on a spatial explicit level and broad coverage. We started our research based on a detailed study assessing impacts of thermal release of a specific nuclear power plant in Switzerland and extracted the most sensitive parameters to derive a generic model of combined fate and effect model for the US. We created a 0.5 arc degree grid accounting for water temperatures, river flow, river width, flow velocity and distance to sea in order to model fate and effect of thermal emissions from different sizes of power plants. Various data sources are tapped and different interpolation methods are applied to cover points without measured data, allowing accounting for uncertainties. We further investigated uncertainties of the characterization factors by varying input parameters and the model assumptions, including the assessment of the affected species. The thermal impacts show significant spatial variability depending on the water body where the cooling water is discharged and the amount of heat released, because the impacts are non-linear. Furthermore this project illustrates how fate and effect model can be integrated to improve the assessment of spatially differentiated impact assessment considerably.

TU 120
Including the influence of worldwide crop cultivation on ecosystem services in life cycle assessment
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Land use is increasing worldwide leading to changes in the ecosystem. Measuring these changes allows for quantification of the impact that each land use activity causes to the environment. This can be achieved through the quantification of changes in ecosystem services. Ecosystem services include carbon sequestration, nutrient cycling, erosion regulation and biotic production potential. The goal of this research is to develop indicators that estimate changes in ecosystem services due to changes in selected land use activities namely, cultivation of rapeseed, soybean and sugarcane, on a global scale. In Life Cycle Impact Assessment (LCIA) these indicators are known as characterization factors (CFs). For this research we focus on erosion regulation and carbon sequestration. The cultivation of the crops is simulated using the Environmental Policy Integrated Climate model (EPIC). EPIC is a spatial and temporal agri-environmental model which simulates the biophysical impacts on homogenous response units characterized by altitude, slope and soil class. Inputs for EPIC simulations include weather and soil profile data, and information on land use, land cover, and crop management. By simulating processes of crop growth, water and nutrient cycles, EPIC produces, among its outputs, estimates of crop yield, sediment transportation and soil carbon sequestration. The characterization factors will be aggregated to country or biome level. Comparisons will be made between results from different spatial aggregation levels, and different management systems regarding input of fertilizer or irrigation.

RA02P - Approaches for comparative hazard and risk assessment of chemicals

TU 121
Health risk assessment of chlorpyrifos with rice farmers in Vietnam
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Vietnam is an agricultural developing country with over 80 per cent of workers employed in agriculture, mainly rice farming cultivation. Farmers use back-pack reservoirs with hand pumps to apply pesticides, but their knowledge of safety in pesticide application is low. Chlorpyrifos is the most common organophosphate insecticide registered for agricultural use in Vietnam, but health risk assessment of chlorpyriphos use has not been carried out and limited investigation in Vietnam and other developing countries. The objective of this study is to evaluate the health risk of chlorpyrifos exposure to a typical group of rice farmers after application in Vietnam, using a probabilistic approach. Biological monitoring of rice farmers was used to estimate chlorpyrifos exposure from all pathways during application. Urine samples (24 h) were collected from farmers (18), from 1 day prior to application and over 5 days post-application, and then analysed for TCP, the main metabolite of chlorpyrifos, using HPLC-MS/MS. Urinary TCP levels were converted into an absorbed daily dose (ADD) of chlorpyrifos for each farmer. The health risk of chlorpyrifos exposure for the rice farmers was characterised by comparing exposure doses (ADD) with acute guideline doses for chlorpyrifos used by USA and Australian agencies.
Post-application chlorpyrifos ADD of farmers varied from 0.4-94.2 µg/kg/d, with a 50th percentile exposure level of 8 µg/kg/d which was about 80-fold higher than the 50th baseline exposure level (0.11 µg/kg/d) (see Figure 1). In comparison with acute guideline doses, the 50th value of post-application exposure among the rice farmers in Vietnam was over 2 times higher than the acute MRL of chlorpyrifos recommended by ATSDR (3 µg/kg/d) and 1.5 times higher than that recommended by US EPA (5 µg/kg/d), but slightly lower than the acute guideline recommended by Australian NRA (10 µg/kg/d). The 95th percentile for the ADD values of the farmers exceeded the acute dose guidelines by a factor of 10 or more.
Conclusion
This case study in Vietnam has shown that rice farmers are at a high risk of chlorpyrifos exposure and resultant adverse health effects, mostly neurotoxicity.

TU 122
A soil ingestion pilot study of a population following a traditional lifestyle typical of rural or wilderness areas
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The relatively few soil ingestion studies underpinning the recommended soil ingestion rates for contaminated site human health risk assessments (HHRAs) that have been conducted to date assessed soil ingestion in children living in urban or suburban areas of the United States, and to a lesser extent, Europe. However, the lifestyle of populations living in North American urban and suburban environments is expected to involve limited direct contact with soil. Conversely, many populations, such as indigenous and Aboriginal peoples residing in rural and wilderness areas of North America and worldwide, practice traditional land use, and participate in activities that increase the frequency of direct contact with soil. Qualitative soil exposure assessments to estimate ingestion rates for Aboriginal populations inhabiting wilderness areas suggest that high levels of soil ingestion (i.e., 400 mg d-1 quantities) may occur, and these levels are many times greater than those recommended by regulatory agencies for use in HHRAs. Accordingly, a study of subjects selected from a wilderness community in Canada was conducted using mass balance tracer methods to estimate soil ingestion and the results compared with previous soil ingestion studies and regulatory guidelines for the soil ingestion rates used in HHRA of contaminated sites. A pilot study of 7 subjects belonging to the Xeni Gwet'in First Nation community living in the Nemiah Valley of British Columbia was conducted over a 3-week period. The study provided the the first quantitative soil ingestion estimates of an Aboriginal population following a traditional or subsistence lifestyle. The mean soil ingestion rates estimated in this study were higher than those currently recommended for HHRAs of adults, and higher than those obtained in most previous studies of adults. However, the soil ingestion rates measured were much lower than earlier qualitative assessments of subsistence lifestyles.

TU 123
Health risk assessment and economic damage due to environmental pollution in a large industrial centre
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A health assessment in Perm, an industrial city in European Russia, has been performed using the WHO methodology. The findings indicate spatially differentiated chronic inhalation exposure of 95% of the Perm citizens to various chemicals (formaldehyde, ethyl benzene, benzene, nitrogen dioxide, etc) at concentrations exceeding maximum permissible concentrations (MPC) (acute exposure) and maximum permissible average daily concentrations, by 16.7 and 13.5 fold, respectively.More than 65% of the exposed population use drinking water which does not meet the standards, i.e. trichloromethane, residual chlorine, iron, manganese and other impurities levels exceed MPC by 2.3 fold. We have observed an increase in acute and chronic hazard indices up to 30 fold for 14 out of the 17 analysed critical body systems and organs. Almost all of the city population is at an unacceptable environmental risk for respiratory, eyesight, hematological and immune disorders as well as central nervous system impairments.More than 173,000 Perm children (99.4%) and all of the 809,000 Perm adults are at an unacceptable carcinogenic risk. The main risk factor is inhalation exposure. The presence of the above mentioned risks has been proven by the identification of contaminants in the blood of the exposed subjects and the deterioration of laboratory, clinical and functional health parameters.We have revealed a true relationship between elevated blood levels of acetaldehyde, formaldehyde, manganese, nickel, chromium, etc. and blood, immune, hepatobiliary disorders and other health effects in the risk group children. We have determined reference levels of the chemical compounds in blood, i.e. acetaldehyde - 0.049 mg/dm^3, formaldehyde - 0.059, benzene - 0.0013, manganese - 0.039, chromium - 0.021, nickel - 0.075 and chloroform - 0.0021 mg/dm^3. We have identified NOAEL values in the air: Ni - 0.00009 mg/m^3, Cr - 0.0001, Mn - 0.00005, formaldehyde - 0.003, acetaldehyde - 0.002, benzene - 0.03 mg/m^3; chloroform reference dose in drinking water is 0.009 mg/(kg x day). We suggest that priority environmental factors (Ni, Cr, Mn, formaldehyde and acetaldehyde) cause 3,246 additional new cases of respiratory diseases. The total economic damage for respiratory diseases is € 1.3 million per year. Acceptable risk levels can be reached by reducing industrial emissions of 19 industrial companies, using traffic optimisation and clean fuel and improving the water supply system quality and water treatment.

TU 124

Snail watch: a tool to assess the risk of metal transfer taking into account their bioavailability

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Current evaluation of soil quality, generally based on physico-chemical characteristics, does not integrate the biological aspect and so the risk of metal transfer to organisms. The use of bioindicators for soil quality assessment allows evaluating the bioavailability of metals and then their risk of transfer.
The aims of this study are firstly to determine the internal concentrations of reference (CIRef) of metallic contaminants (Cd, Pb, As, Cr, Cu and Zn) in Cantareus aspersus snails. Then, CIRef are used to identify abnormal metal transfer to snails. Secondly, to estimate the soil characteristics influence on metal accumulation using multivariate equation. On these basis, our last objective is to establish procedure to Evaluate the RIsk of Transfer of MEtals (ERITME) to snails to determine management priorities that take into account physico-chemical and biological parameters of metal bioavailability.
CIRef have been established on the basis of concentrations in the snails exposed on unpolluted sites for 28 days i.e. snail watch. In snails exposed on other sites, when the value of the median of metal concentrations is above the CIRef, the site will be considered as presenting abnormal risk of metal transfer. Most of the time, the use of these CIRef has allowed to identify the contaminated sites. They also reveal unexpected metal transfers on reference sites and a lack of transfer on contaminated sites. The use of multivariate equations has enabled to determine pH, organic carbon content and iron oxides as parameters influencing metal bioavailability to snails.
The ERITME tool, based on CIRef, allows to highlight the risk of transfer of each metal to snails not based on total metal soil concentration but on a biological risk assessment. A hierarchy between sites can be established using the total accumulation quotient (TAQ: mean of AQ, with AQ = [Csnail]/[CIRef]). TAQ represents the global risk of metal transfer of a site. The ranking of the TAQ emphasize the transfer risk of industrial sites. On these sites, As, Cd and Pb transfers have been identified, putting these sites on top of the management priorities. A risk of Pb and As transfer on forest modalities that are slightly or not polluted by these metals was also evidenced, highlighting the need to use biological tools that take into account metal bioavailability influenced by soil characteristics for risk assessment procedures.

TU 125

Prioritising chemicals for EQS development

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Environmental Quality Standards (EQSs) are a key tool under the Water Framework Directive (WFD) to help assess the chemical status of water bodies. However it is important that EQSs are developed for substances that pose the greatest risk to the aquatic environment, and that emerging contaminants are adequately considered.
Various methods for prioritising chemicals have been described, incorporating both exposure and effects. Approaches that prioritise on the basis of risk often require significant inputs, even a draft EQS, or detailed exposure modelling. We describe a simple approach to prioritisation that requires only modest inputs and assigns chemicals to different risk categories.
Unlike more sophisticated schemes, this method does not require a draft EQS in order to prioritise chemicals. Rather, it categorises chemicals in terms of their likely (or actual) environmental occurrence based on use and monitoring data, and the hazard they pose to aquatic life, based on persistence, bioaccumulation and toxicity criteria. Classifications based on exposure and hazard are then combined and chemicals assigned to risk categories. Those assigned to the highest risk category are taken forward as potential candidates for EQS development.
Almost a third of the chemicals considered could not be assigned a risk ranking due to a lack of data. This is of particular concern for emerging contaminants where both hazard and exposure data are often limited. Lack of exposure or hazard data contributed equally to the assignment of 'Insufficient information'. Predictive tools such as QSARs or 'read across' techniques may have a useful role in filling some gaps in hazard data. Gaps in exposure data may be filled by gathering monitoring data; in this respect the tool has helped identify monitoring needs that may lead to identifying future EQS candidates.
The prioritisation methodology is not suitable for metals and other inorganic chemicals because of biases in the way that fate and behaviour are dealt with e.g. measures of degradation and bioaccumulation. A separate approach for such chemicals may need to be developed.
Finally, it is important to peer review the outputs of the scheme because this can help introduce relevant new data or information about significant trends in likely exposure (e.g. planned restrictions on use) that will affect the chemical's priority.

TU 126

Klimisch 2.0 - raising the bar to increase the scientific quality of environmental risk assessments

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Ecotoxicological data with high reliability and relevance are needed in different European legislations and regulations, e.g. REACH, EMEA or the Water Framework Directive, to guarantee the scientific quality of environmental risk assessments for chemical substances.
Criteria developed by Klimisch et al. (1997) are widely used to assess data quality. This assessment relies heavily on the completeness and quality of the data set and conclusions often require expert judgement, which is sensitive to scientific and cognitive variations. Assessments based on data with low reliability could lead to incorrect conclusions and may underestimate risks in the environment or lead to unnecessary risk mitigation measures being introduced.
Several recent studies (e.g. Duchemin et al. 2010, Durou et al. 2011, Junghans et al. 2011a,b , Ågerstrand et al. 2011a) have shown that the Klimisch system is not accurate and prescriptive enough to guarantee consistency between different risk assessors. This presents a general problem in the data quality evaluation of all chemicals, and is not limited to specific substance groups.
We therefore recommend to amend the Klimisch system by adding a scoring system for the criteria for reliability and relevance of the data, to ensure a more consistent evaluation between assessors. The detailed methods developed by Kuester et al. (2009, 2010) and Ågerstrand et al. (2011b) provide a useful basis for refining the quality assessment criteria and could be adapted for all substance groups. Additionally, the assessment should also consider if a critical study is plausible in the context to the whole dataset. To test plausibility, we propose that critical information distributed across different studies should be evaluated with a weight of evidence assessment, e.g. Gross et al. (2011).
In conclusion, we propose a more structured reliability and relevance evaluation of critical ecotoxicolgical studies together with a plausibility assessment based on a weight of evidence approach for the whole dataset. This should increase the scientific quality of environmental risk assessments of substances, e.g. in Environmental Quality Standard (EQS) derivation according to the Technical Guidance Document for EQS (European Commission 2011).
The intention is to provide guidance for different interests from science, hazard assessment and regulation. However, we recognize that the weighting assigned to criteria might vary between different chemical assessment regulations.

TU 127

EQS derivation for metals in EU and USA: a comparison using copper

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Standards and targets are derived to help us take action to protect and improve water quality. However, environmental agencies throughout the world have or will develop frameworks that may differ substantially therefore resulting in different standards for the same pollutant. This presentation aims at providing an overview of the frameworks developed in the EU and USA for the derivation of EQS for metals. Central focus will be on differences in approach used for the development, validation and implementation of the Biotic Ligand Models (BLM). Indeed, in the USA acute BLMs are proposed for 2 different trophic levels (invertebrates and fish), while in the EU chronic BLMs are used for 3 different trophic levels (algae, invertebrates and fish). Once normalized for bioavailability safe threshold concentrations are calculated as the lower 5th percentile of the whole species sensitivity distribution SSD in the EU while only the 4 most sensitive genus mean values are used to derive the 5th percentile using least square regression analysis. The influence of such differences in both frameworks on the EQS setting will be illustrated using Cu as an example. EQS for different surface waters (with different bioavailability) will be demonstrated. In most cases, it seems that the differences in EQS values between both frameworks are within a factor of 2-3. Therefore, although there is a general tendency to incorporate such concepts in regulatory frameworks for metals there is a clear need for harmonization of frameworks as highlighted by the OECD.

TU 128
Efficient toxicity testing when test information is uncertain: a stochastic cost-effectiveness analysis
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Changing policy settings for chemicals' risk management, for example in Europe and in the U.S., have led to increasing awareness of the urgent need for more efficient testing of chemicals. The economic concept of efficiency calls for allocating scarce resources (e.g. money, time, human skills) such that output is maximised. Hence, if for a toxicological endpoint different testing options exist, a decision-maker must identify the testing strategy that provides the highest information gain per unit of cost. This, clearly, requires to weigh the costs associated with a testing strategy, usually measured in monetary terms, against the information gains from testing. The latter are commonly expressed in terms of the a test's predictive performance. Cost-effectiveness analysis (CEA) provides a powerful tool for solving this problem. CEA allows for a comparative evaluation and for identifying trade-offs between costs and information gains for different testing methods and strategies. While CEA has become widely used in many domains, for example in medical decision-making, there are only few attempts to apply CEA to testing in toxicology. These studies share the assumption that information gains from testing, measured in terms of a test's predictive performance, are known. The predictive performance of testing methods is, however, uncertain, irrespective of what type of testing method (e.g. a "gold standard"in vivo test, an in vitro test, or a "non-testing"method) is used. Our study, therefore, moves beyond existing deterministic CEA modelsused in toxicology by developing a stochastic approach to CEA that accounts for the uncertainty about the quality of test information. The aim is to allow for a more robust and transparent evaluation of testing methods and strategies both within and across endpoints. This guides decision-makers in coherently selecting the testing method or strategy that provides the highest information gains per unit of cost. Another contribution of the study is to offer a critical synopsis of data and research needs in order to apply our approach to various endpoints and to make it operational in the regulatory context of REACH.

TU 129
IFRA Environmental Standards: risk and hazard assessment update for 2012
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The International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environmental Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the developing IFRA Environmental Standards, reported here are the results of these additional tests (for both risk and hazard assessments). These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented. IFRA and RIFM have decided to present an annual update of this test program and the IFRA Environmental Standards. This work represents the latest effort for 2011-2012.

TU 130
The HEROIC project: coordinated efforts towards the harmonization and cross-fertilization of human and environmental risk assessment of chemical substances
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Today, human risk assessment (HRA) and environmental risk assessment (ERA) are typically separated. There is a lack of mutual understanding between experts and data from toxicological and ecotoxicological studies are not readily accessible by risk assessors of the two disciplines. The need for RA will continue to increase (e.g. REACH or toxicity of mixtures) along with budget restrictions and political and public pressure to reduce the number of animal tests. Therefore more cost effective, predictive and rapid tests for high quality sustainable RA are needed, including a better exploitation of existing data.
The HEROIC project - a coordination action of the 7th FP - will provide a platform for networking among all the relevant stakeholders in the RA value chain and will provide them with the most relevant background information to contribute to the development of harmonised approaches which meet the challenges of RA. The project will enable the improvement and harmonisation of tools and methods in RA, by exploring how data generated in ecotoxicology and human toxicology can be applied across disciplines for integrated RA, and develop a framework for integrated methodologies and approaches for RA. This will increase transparency in RA and allow better risk communication to maintain public trust and to give unambiguous guidance for improved risk management.
HEROIC starts with a comprehensive landscaping exercise to identify common methodological and data needs in current human and environmental risk assessment practices. We will then evaluate existing in-vivo, in-vitro and in-silico methods for hazard and exposure assessment. The selection process ranks and weights data based on their reliability and relevance and uses a Weight-of-Evidence approach to integrate such information to develop an Integrated Testing Strategy (ITS) for decision making. A dedicated web portal called 'Tox-Hub' that presents information from diverse sources and that functions as a central point of access to the most relevant toxicological and ecotoxicological information will be created. A diverse range of dedicated activities is planned for information, dissemination, capacity building and communication.
These coordinating activities will result in enhanced sharing of knowledge, building consensus and development of clear, easily understood, transparent and unambiguous integrated RA procedures.

RA03P - Are Environmental Specimen Banks ready to face future challenges of environmental chemistry and regulatory toxicology?

TU 133
Fish from the German environmental specimen bank - patterns, relations and time trends of PCDD/F and dl-PCB in breams (abramis brama) from German rivers
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Environmental specimen banks (ESBs) are collections of samples which contribute widely to the understanding of connections within regional as well as global ecosystem behaviours. Furthermore they deliver valuable understandings not only of retrospective time lines but also of similarities and differences within the collected information as well as giving the opportunity for discussing trends.
The German ESB is one of the largest specimen collection programmes worldwide, monitoring the persistent and highly toxic polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) and dioxin-like PCB (dl-PCB) in aquatic ecosystems in order to evaluate the overall state of environmental protection within the tributary system of the main rivers in Germany. Serving this purpose, the common bream, abramis brama, has been selected as a specimen for this monitoring. It is common, widespread, trophically reasonably high and easy to sample and also forming a link towards human consumption.
We present actual time trends and levels for PCDD/F and dl-PCB in breams from seven major rivers in Germany between 2003 and 2008. Levels are notably going down for PCDD/F concentrations but for dl-PCB there is a non-uniform overall picture showing rather a baseline than a clear decrease at fairly high levels. Generally, for both parameter groups concentrations are at a level which is of long-term concern for environmental safety as well as human health perspective, e.g. compared against actual maximum levels from EU feed/food legislation. This is especially true for the lower courses of the big streams Rhine and Elbe.
Furthermore, the single compound data for the analyte groups give additional understanding of pattern of PCDD/F and dl-PCB as well as their correlation. The presented data show for example the differences of ratios between those two groups as being fairly unique for the sampled river system. Discussion could point as well towards different or similar industrial influences of the different tributary system as towards unification of pattern, e.g. by metabolic influences.

TU 134
A 10 yr retrospective study of mussel condition in North Iberian Peninsula based on the 'mussel shell section' of the Biscay Bay Environmental Biospecimen Bank
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An environmental specimen bank (ESB) is an archive for biological samples that can be used to perform (traditionally chemical) analyses to obtain information about long-term variability and temporal trends in the chemistry of the biota and the ecosystem quality. Aware that chemical endpoints alone are not enough for ecosystem health assessment, the Biscay Bay Environmental Bioespecimen Bank (BBEBB) was designed including specimens for biological endpoints, say biopsies for histopathology/ immunochemistry, cryo-specimens for molecular/cellular analysis, and dried mineralized tissues (shells/otoliths) for biometric determinations. Banking methods and specimen sampling and processing are being adapted for these needs. In a first practical application, a retrospective study was performed using the 'Mussel Shell Section' of the BBEBB (2001-2010; 22 localities covering the area of geographical distribution of *Mytilus galloprovincialis* in the North Iberian coast). Changes in shell size (length, L; width, W; height, H), allometry and shell weight (SW) were analyzed, and the shell thickness was estimated after regression of the log SW against SSF (Shell Size Factor, LxWxH in mm). Overall, shell growth exhibited a decreasing trend during the study period with a seemingly more marked arrest growth in 2003-2006. These results are discussed in terms of interactions between the global environment trends and the consequences of the Prestige oil spill. However, since only specimens of L=3.5-4.5 cm were analyzed in most cases and mussels of the same shell length can belong to different age classes depending on the geographical variability and the influence of environmental conditions for a given locality, the incidence of the age in the interpretation of the obtained results is also discussed. In summary, the development of Mussel Shell Sections in environmental biospecimen banks is highly recommended as they may offer an excellent opportunity for retrospective monitoring after low cost processing and storage. In addition, our results also suggest that mussel watch monitoring programs and ESB archives should be designed on the basis of samples of same-age rather same-length mussels. This study was supported by the Government of the Basque Country through K-EGOKITZEN project.

TU 135
Using banked seabird eggs for determining geographic patterns of trace elements in marine regions
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Selenium and arsenic occur naturally in the environment but they are also known to be toxic in excess of trace levels. During the 1990s, elevated concentrations of arsenic were reported in liver tissue of ringed seals taken by Alaska Native hunters in Norton Sound, Alaska. To determine whether eggs of colonial seabirds might be good monitoring matrices for both arsenic and selenium in marine regions of Alaska, total arsenic (As) and selenium (Se) mass fractions were measured in 78 seabird egg samples collected in 2008 and banked at the US Marine Environmental Specimen Bank (Marine ESB). These eggs were collected from several colonies located within Norton Sound as well as long-term monitoring sites in two other major Alaska marine regions, St. Lazaria Island in the Gulf of Alaska and St. George Island in the Bering Sea and were from three species: common murre (Uria aalge), thick-billed murre (U. lomvia) and glaucous gulls (Larus hyperboreus). Egg samples were analyzed using collision cell kinetic energy discrimination inductively coupled plasma mass spectrometry (ICP-MS). Total arsenic mass fractions ranged from 0.015 µg/kg to 0.320 µg/kg while the levels of Se ranged from 0.411 µg/kg to 1.017 µg/kg. As and Se levels were higher in most of the samples collected from the Norton Sound colonies compared to other colonies. Norton Sound is located in a highly mineralized region of Alaska and is an area of historical gold-mining that continues today and could be a contributing factor to the bioaccumulation of arsenic and selenium in this region. In addition, As mass fractions were also measured in 43 common murre and glaucous gull eggs collected in 1999, 2000, and 2005 from colonies located throughout the Bering Sea, including 4 of the same colonies as those collected in 2008. The As mass fractions ranged from 0.060 µg/kg to 0.349 µg/kg and were also more elevated in the Norton Sound colonies than other colonies in the Bering Sea. The data reported here illustrates that seabird eggs can be a useful monitoring tool to help determine geographic patterns of trace elements in marine regions, specifically the sub-Arctic regions of Alaska.

TU 136
Specimen security in long term specimen banking - zebra mussel example in the German ESB
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In the late eighteenth century, the zebra mussel (Dreissena polymorpha) established itself as an invasive species - sometimes in huge numbers - in large parts of Europe and North America. These new settlers provided an excellent opportunity for environmental observers to use an effective plankton filter to indicate bioavailable and bio-accumulative anthropogenic contamination in many areas. The successful invasion of the zebra mussel can be seen as the trigger for a multitude of "mussel watch"programmes in limnic waters of Eurasia and North America, and has also led to the zebra mussel's permanent presence in the German Environmental Specimen Bank (ESB) for the last 20 years.
In the past few years many areas have seen a dramatic drop in zebra mussel numbers. Now, out of nowhere, what was once a successful model for monitoring waters is endanger of not producing a sufficient number of specimens for large samples. A possible cause is the receding eutrophication level of the waters and the resulting lack of food. Another invasive species, the quagga mussel (Dreissena bugensis), which has a stronger presence in many areas, is increasing the pressure on the zebra mussel populations too. Recent research has also shown that fertile hybrids between D. polymorpha and D. bugensis are possible in the wild - making biomonitoring even more difficult. Due to different filtration rates, it must be assumed that accumulation in both species is also different, which is why D. bugensis and hybrids of both species have to be excluded from the results of monitoring studies using D. polymorpha for them to be comparable.
Investigations into the current significance of the zebra mussel in long-term monitoring projects and environmental specimen banking programmes in the German ESB show the specific information the zebra mussel provides. This makes clear that, alongside the common bream (Abramis brama) and suspended particulate matter, it is an important component of the limnic sample set. Loss of this sample type would result in a huge loss of information.
Therefore, the most important task concerning D. polymorpha as a biomonitoring tool is to:
- find out the differences in how substances accumulate in D. polymorpha and D. bugensis;
- discover whether there are wild hybrids; and
- develop a method to differentiate unequivocally between both species for large samples.

TU 137
Organic compounds in suspended particulate matter - results from the German environmental specimen bank
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Since 2005 suspended particulate matter (SPM) in the river systems of Rhine and Elbe has been sampled, processed and archived in the frame of the German Environmental Specimen Bank (ESB). Sampling is carried out with sedimentation boxes which are emptied monthly. At the end of the year monthly samples are lyophilized and pooled to a homogenate. From every homogenate about 200 subsamples are produced and stored in the ESB. The whole processing is conducted under deep frozen conditions. Some sub-specimens are chemical characterized for inorganic substances, CHCs and PAHs.
The results of the five years period 2005 -2009 show considerable differences between the two river systems. The concentrations of PAH16 as well as of PCB6 and DDX increase downstream the river Rhine whereas in the Elbe system only few differences of concentration are observed between the single sampling sites. One exception is the sampling site Elbe/Blankenese near Hamburg harbor which represents the passage of the Elbe into the North Sea and is tidal influenced. Here, lowest concentrations for all substances are observed. For the most substances the concentrations at Blankenese are less than 20 % of the concentrations at the other samplings sites. This is probably caused by dilution of the Elbe water with salt water of the North Sea.
With exception of Elbe/Blankenese concentrations for most substances are significantly higher in the river Elbe compared to the river Rhine. Especially the concentrations of DDX and HCHs are much higher in SPM from the Elbe than in SPM from the Rhine.
Within the observation period significant reductions of B[a]A, B[e]P and B[a]P concentrations in the river Rhine could be observed.

TU 138
Standardized freshwater mussel watch for monitoring of aquatic environments: implications and possibilities
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Mussel Watch is one of the most effective approaches to study the dynamics of aquatic pollution. Unfortunately, it utilization is usually limited to the depending on collection of wild mussels. Based on our pilot studies, we recommend to establish an innovative "standardized Freshwater Mussel Watch"system. Namely, a population of "standardized"biological indicator mussel (e.g., Anodonta woodiana) is first established by artificial propagation techniques, which possesses the similar biometric characterization, genetic quality, and low background of environmental pollution. Subsequently, the standardized mussels with the same size will be transplanted to some of typical fishery waters (e.g., Taihu Lake) as the 'experimental groups', and be continuly reared in the artificial propagation pond as the 'control groups', respectively. Consequently, a comparative assessment on temporal and spatial dynamics of pollution and bioaccumulation of persistent toxic substances (e.g., heavy metals, organic pollutants, natural toxicants) can be investigated with system error at a much smaller level and without destroying the wild mussel population. At the same time, a living individual pool of glochidia, juvenile, and adults can be established for exposure and toxicological studies, in order to assure the reliability of the field works and understand the corresponding impact degree,tolerance mechanism, and stress threshold.The present study will make it possible to setup the world's first 'Freshwater Mussel Watch' system, the standardized living individual pool, and environmental specimen bank of Anodonta woodiana for special use on conservation and investigation of freshwater fishery environments.

RA06P - Contaminated sediments in a changing environment

TU 140
The impact of high water discharges on sediment quality in the Elbe estuary
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Contamination of the Elbe estuary with particle bound compounds mostly (with the exception of TBT which had its source in the Hamburg Harbour) derives from "areas of risk"upstream of Hamburg such as the Czech Republic for organic contaminants like HCH, HCB, PCB, DDX and TBT, the tributary Mulde for As, Dioxins and HCH, and the Saale for a number of heavy metals like Cd, Hg, Cu, Zn, and Pb. The major transport of contaminants happens during periods of high water discharge, when historic contaminated soil or sediment in the catchment is resuspended during flood events and transported downstream. For some compounds, the yearly load of particle bound contaminants is transported during a few days of a high water discharge (Heise, Krüger et al. 2008). Up to now, the impact of contaminants on the quality of sediments in the estuary as a consequence of a high discharge event, however, has not been very well investigated.
The Elbe estuary is characterized by the Hamburg Port, the second largest port in Hamburg, about 100 km upstream of the mouth of the river. Even though the river downstream of Hamburg has been maintained as a deep water navigation channel, 94 % of the area is ecologically protected by FFH and Birds directive. Some of the ecologically valuable freshwater wadden areas are close to Hamburg, and at the mouth of the Elbe the river widens to a large marine wadden area which is a feeding and nesting site of many bird and fish species.
In order to assess the impact of a high water discharge situation on the sediment quality of ecologically important wadden areas, freshly deposited sediment (FDS) was sampled during normal and high water discharges at two freshwater mudflats close to Hamburg and at two places near the mouth of the Elbe close to the North Sea. Chemical and ecotoxicological analyses were carried out on the sampled material. Elevated discharges did not lead to any significant increase of contamination of FSD in either sampling site. An extreme high water event in January 2011, however, led to an increase in contamination in freshwater as well as coastal mud flats. Especially high was the impact on the more upstream "Heuckenlock"which is of particular ecological importance. Ecotoxicologically, little effect could be measured and it needs to be clarified how bioavailable the historic contaminants still are after their transport downstream the Elbe River.

TU 141
Toxicity of suspended matter and sediments in relation to contaminant load and expected effects of climate change on European rivers
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Climate change may not only affect river water discharges in terms of water quantity, but also in terms of water quality. During flooding periods, for instance, higher water discharges may cause a dilution of normal pollution levels, while on the other hand, higher discharges may also result in an increased mobilization of polluted sediments and in surface run-off from possibly polluted flood plains and inundated urban areas. During high water episodes huge increases of contaminant fluxes may be recorded. Long term projections of regional climate models are variable and tend to predict more exteme rainfall and dry episodes but with a limited overall increase in river discharges and suspended particulate matter (SPM) loads to the North Sea. To study the possbible impact of climate change on the quality of urban and coastal waters, a battery of in vitro bioassays was applied to determine the toxicity profiles of environmental samples collected during climate change related events. SPM and sediment samples were collected in rivers from Norway, Sweden, Denmark, Germany, and The Netherlands during periods of dryness, flooding and extreme run-off. In addition, passive sampling was performed using silicone rubber sheets to evaluate the effects on dissolved contaminants. Extracts from SPM and passive samples were tested for respiratory toxicity (Microtox), dioxin-like activities, (anti)androgenic, (anti)estrogenic, and thyroid hormone-like activities, and for mutagenicity. Variable patterns of both increased or decreased toxicity in relation to discharge conditions were noted between rivers and different years within rivers. Marked increases of toxicity were noted for episodes of melting of 'black snow' (Akerselva river, Oslo) and overflow of mixed sewer systems (Harrestrup river, Copenhagen). Contaminant concentrations of SPM and sediment were classified based on a toxic-unit approach and will be compared to the in-vitro toxicity profiles. Results of multivariate statistical analysis (PCA, cluster analysis) and different toxicity ranking and classification approaches will be presented. The results will be be included in a web-based communication tool (Open Earth) open to the general public. The work described was performed within the context of the DiPol project (Impact of Climate Change on the quality of urban and coastal waters - Diffuse Pollution), which is funded by the Interreg IVB North Sea Region Programme.

TU 142
The effects of CO2 leakages from marine stable geological formations on the mobility and availability of metals
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Carbon dioxide capture and storage (CCS) in marine sub-seabed geological formations, is already contemplated in international conventions as a mitigation measure to reduce the concentration of CO_2 in the atmosphere. Although this technology is currently considered to be technically feasible there is lack of information on the environmental impacts of accidental leakage. CO_2 Seepage is likely to create a considerable pH decrease in the sediment, sediment pore water, sediment-water interface and finally in the water column. Increase in acidity will lead to several biogeochemical alterations that can result in detrimental effects in the ecosystem. Changes in the solubility, speciation, mobility and bioavailability of trace metals is expected as a direct result of pH decrease

Laboratory-scale experiments were performed, involving direct release of carbon dioxide into sediment, inside non-pressurized chambers, in order to provide data on the possible effects of CO_2 leakage from geological storage sites on the fate of several metals. Marine sediments from two contaminated sites located in the south of Spain were collected and submitted to acidification by means of CO_2 injection. The test lasted 10 days and samples were collected on the beginning and end of the experiment for metal analysis. The results revealed that mobility of metals from sediment to water column depended on the site, metal and time. Metals concentrations generally increased with time of exposure to CO_2 injection. Within the metals tested zinc, copper, cobalt, lead and iron were most greatly influenced by acidification.

TU 143
Changes on the biogeochemistry of synthetic surfactants from river to marine sediments
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Aquatic systems are subjected to the influence of both urban and industrial wastewater discharges, which are among the main sources of organic contaminants. Due to their extensive use in a wide variety of applications, significantly high concentrations of surfactants can be found in wastewater. Once they reach water bodies, a significant fraction of these compounds can be detected in sediments due to their moderate sorption capacity. It is often assumed that surfactants are well preserved in the sedimentary column once anoxic depths are reached, which can happen within a few millimetres to centimetres depending on the aquatic system. In this work we have studied the diagenetic fate of the most commonly used surfactants: alcohol polyethoxylates (AEOs) and nonylphenol polyethoxylates (NPEOs), both non-ionics, and linear alkylbenzene sulfonates (LAS) and alkyl ethoxysulfates (AES), both anionics. Their concentrations in sediment and pore water were determined along the sedimentary column by sampling cores in three different aquatic systems from SW Spain: the salt-marsh environment of the Bay of Cadiz, the middle stretch and the estuary of the Guadalete River, and the Bornos water reservoir. Different distributions were observed according to the respective uses, production volumes and physicochemical properties of each surfactant. Levels of non-ionics (up to 12 mg/kg) were twice as high as to those for anionics near industrial areas and ports, whereas the opposite was found near urban wastewater discharge outlets (up to 18 mg/kg). The most remarkable result was that sulfophenyl carboxylic acids (SPC), LAS degradation products, were identified in anoxic pore water. However, this was observed only in marine, and, to a minor extension, estuarine sediments. No SPC were detected in sediments from freshwater systems. The presence of sulfate-reducing bacteria in marine sediments (or absence in freshwater environments) seems to be strongly related to the degradation process. On the other hand, no changes in the average length of AEO and NPEO ethoxylated chains were observed along sediment cores from any type of aquatic system, suggesting that their biodegradation is very limited in anoxic conditions. This may be directly related to their lower bioavailability too, as their calculated sediment - pore water distribution coefficients showed much higher values when compared to those for most polar anionic surfactants (e.g., 553 ± 98 for NPEOs vs. 11 ± 7 for AES).

TU 144
Historical trend and pollution assessment for selected trace elements in sediment cores from the Orbetello lagoon (Tuscany, Italy)
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Heavy metal enrichment in sediment may be due to both natural and anthropogenic contribution, which may be recognized by studying the concentration profiles along depth in sediment cores. A study on the different contribution due to heavy metal in the sediment was carried out in the Orbetello lagoon (Tuscany, Italy). The lagoon is located within a volcanic district, characterized by hydrothermal ore deposits and natural positive geochemical anomalies for some heavy metals may occur. Consequently, the correct interpretation of natural and anthropogenic enrichment is a main issue for a correct environmental impact assessment. In this work, selected trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) were analysed in two dated sediment cores (OR96 and OR98), about 2 meters long, and the non-dimensional Enrichment Factor (EF) was calculated for each element using local background concentrations. The determination of Cd, Cu, and Zn concentrations demonstrated in both the cores an upper anthropogenic enrichment referable to the last half of the 20th century, while Pb contamination was found to begin earlier, during the first half of the last century. In spite of considerable EFs showed by these elements (Pb and Cd EFs up to 6-7) such contamination do not determine environmental concern, because concentrations exceed in few cases the limits adopted by international sediment quality guidelines. As regards Hg concentration, a recent contamination, referable to the last few decades is clearly distinguishable only in one core. Conversely, a marked Hg positive geochemical anomaly dated around 1850 present in both the cores was considered as a result of specific anthropogenic activity related to the extensive mining activity and metallurgical processes in the region, which started in the mid-nineteenth century. On the contrary, the significant enrichment recorded for As in lower levels of core OR96 were interpreted as the results of increased contribution from the Albegna River catchment, were high As content is present in ore deposits of Hg and Sb. Among the investigated heavy metals only Hg, may be considered element of concern, because the measured concentrations exceed, with few exception, the reference values of sediment guidelines. For this reason, indication to investigate potential toxicity through studies on mercury bioavailability and ecotoxicological response arises from the present research.

TU 145
Rapid contact kinetic assay with Vibrio fischeri in the assessment of small streams sediments in the Czech Republic
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Small streams form an essential part of aquatic networks in all river basins but they are negatively affected by various anthropogenic pollutants. The monitoring of water quality is commonly based on chemical analyses of selected priority contaminants but water and sediments in small streams contain complex mixtures of different chemicals classes, which may cause various toxic effects to aquatic biota. Biological assays thus serve as a complementary tool in the effect-based monitoring of water or sediment quality. In the present study, we investigated 25 localities from two different regions in the Czech Republic. Sediment samples from each locality were repeatedly collected for two years with the main goal to compare outcomes of chemical and biological/ecotoxicological analyses. Chemical analyses assessed levels of toxic metals, PAHs and POPs (PCBs, OCPs), and sediment toxicity was determined using a kinetic assay (Flash Test) with the luminescence bacterium Vibrio fischeri. We have observed significant differences between both studied regions in the Czech Republic as well as between different sampling periods. Statistical analyses repeatedly revealed relatively poor correlations between chemical contamination data and toxicity, while total organic carbon (TOC) appeared to be related to observed toxic effects. The present study shows successful application of the kinetic V. fisheri test in the effect-based monitoring of sediments demonstrating thus complementarity of both chemical and ecotoxicological data.

TU 146

Influence of feeding type and behaviour on whole sediment toxicity tests with the oligochaete Lumbriculus variegatus

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Lumbriculus variegatus were exposed for 28 days to 0, 180, 560 and 1800 µg Zn g-1 sediment and received 2 feeding regimes. The partitioning of Zn between sediment, pore water and overlying water was enhanced by a 2-year equilibration period. Feeding regimes used in the toxicity tests were (i) food (Urtica+cellulose) spiked into the sediment with no additional feeding during the exposure (SED+) and (ii) daily feeding with TetraMin (TetraMin). This 4x2 design experiment was set up to test the hypothesis that feeding characteristics can affect the performance, response to toxicants and internal toxicant distribution of L. variegatus.
No adverse effects were noted in the TetraMin treatment, while in the SED+ treatment the total number of worms and biomass were significantly reduced at 560 µg g-1 and 180 µg g-1, respectively. For both feeding regimes, Zn body burdens were significantly increased only at 1800 µg g-1 (SEMZn-AVS>0). Overall, the results show that despite similarity in Zn bioaccumulation between two feeding regimes, biological responses were only noted in worms fed via the sediment. The lack of toxicity in the TetraMin treatment was attributed to selective feeding on TetraMin and to daily feeding regime, which promoted the organisms to spend less time in the sediment thus reducing Zn exposure. Significant Zn bioaccumulation not accompanied with biological effects at 1800 µg g-1 suggests internal detoxification or other regulation mechanisms. In the SED+ treatment, significant biological effects not accompanied with Zn bioaccumulation were observed at 180 and 560 µg g-1. Feeding inhibition resulting from an avoidance reaction to contaminated food/sediment or different mechanisms of toxicity due to dietary Zn, were assumed to be the causes. The internal Zn distributions were analysed using synchrotron radiation based confocal micro X-ray fluorescence (XRF). The results show that in worms fed via the sediment, Zn is mainly associated with epidermis and less with the gut walls. It is, however, equally present in gut wall and epidermis in worms fed TetraMin. This supports the assumption of feeding inhibition due to avoidance behaviour toward contaminated sediment/food. The XRF data also show that at 1800 µg g-1, Zn was mainly distributed in the epidermis and sub-epidermis of L. variegatus, confirming the role of dissolved Zn as the predominant exposure route when SEMZn-AVS>0.

TU 147

Toxicity of organic micropollutants in the Yangtze Three Gorges Reservoir - MICROTOX

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The creation of the Three Gorges Reservoir caused the flooding of former urban, industrial and agricultural areas. Consequently, substantial amounts of organic and inorganic pollutants were released into the reservoir. Beyond contaminants and nutrients enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediment onto agriculturally used areas along the reservoirs shore.
In order to assess (i) possible links between molecular/biochemical responses and ecologically relevant effects, and (ii) if ecotoxicological effects might be related to adverse effects in fish from the field, sediment samples and fish were collected at selected locations and analyzed using a weight-of-evidence (WOE) approach with several lines of evidence. The objective of the poster presentation is to introduce the conceptual framework and to present first results of the ongoing study.
As previously addressed by Chapman & Hollert (2006) a variety of lines of evidence can be used in WOE studies. Briefly, (i) a comprehensive battery of acute (neutral red assay, *Arthrobacter*, fish embryo toxicity test and sediment contact assay with *Danio rerio*) and mechanism-specific bioassays (micronucleus assay and Ames test, EROD and YES assays) is applied to characterize the ecotoxicological hazard potential. (ii) Biochemical investigations and the micronucleus assay with erythrocytes will be applied, representing *in situ* parameters as well as (iii) persistent organic pollutants (PCB, PAH and PCDD/Fs), endocrine disrupting substances, limnochemical parameters and the concentration of heavy metals will be analyzed. To identify organic contaminants causing effects in sediment toxicity assays, (iv) effect directed analysis (EDA) will be applied. So far sediment and fish samples have been taken at major cities as well as feeder rivers and their estuaries along the Yangtze Three Gorges Reservoir in order to characterize the state of contamination and the potential risk for human and wildlife. The monitoring strategy should help to observe the water body's quality and to serve as a basis to initiate if necessary counteractive measures.
Reference
Chapman PM, Hollert H (2006) Should the sediment quality triad become a tetrad, a pentad, or possibly even a hexad? JSS - Journal of Soils and Sediments 6: 4-8

TU 149

Toxicity of persistent organic pollutants from Baltic Sea sediments in the fish embryo test and the EROD assay

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The Baltic Sea, one of the largest brackish water bodies in the world, is considered to be an exceptionally sensitive and endangered marine ecosystem. The average residence time of Baltic Sea water ranges between 25 and 50 years, thus facilitating the accumulation of pollutants in sediment. For a deeper insight into the ecotoxicological effects of pollutants in Baltic Sea sediments, biological test systems were applied with exhaustive as well as mild sediment extracts. Extracts from PLE and mild methanol/water extraction, were investigated for their dioxin-like activity and their embryotoxic impact on the development of zebrafish (*Danio rerio*) embryos. PLE extracts were considered to represent the entire toxic potential and the mild methanol/water extracts to contain rather bioavailable pollutants. Applied biotests were the fish embryo test in 96-well plates and the EROD assay with RTL W1 cells. Results from the fish embryo test revealed no effects caused by methanol/water extracts in contrast to the PLE extracts, which gave strong embryotoxic potential. Results on the EROD inducing potential indicated dioxin-like activity for all investigated sampling sites. PLE extracts showed clearly higher dioxin-like activity than mild methanol/water extracts. The study revealed an ecotoxicological burden for all Baltic Sea sediments. Especially the Eastern Gotland Basin, the Gulf of Finland and the Arkona Basin appear highly polluted, but according to the methanol/water extracts, this pollution is not readily available for developing fish embryos. However, the ability of the mild extracts to resemble the bioavailable fraction might be limited. For a comprehensive ecotoxicological assessment it is necessary to thoroughly investigate the bioavailability and to identify the causative pollutants by chemical analyses.

TU 150

Identi?cation of hazard factors and ecotoxicological risks of sediments from the Tietê River Basin (Brazil)

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The Tietê River, selected in this study as an example for a highly contaminated river system, is located in the most important economical center in Brazil, São Paulo state. The reservoirs constructed along its course are widely used for providing drinking water, as water sources for agricultural irrigation and as recreation sites. Several approaches involving different test organisms, several endpoints and different sediment phases (liquid as extract and solid as freeze-dried samples) were followed under laboratory conditions. Results document that sediment samples from various sites along the Tietê River Basin are differentially polluted with contaminants which cause not only acute cytotoxicity, but also genotoxicity and AhR-mediated toxicity in fish cells, as well as embryo toxicity. Moreover, mutagenicity was recorded in situ in fish caught from the field. Since major toxicities were associated to samples from the metropolitan region, sediment samples from this region were submitted to an effect-directed analysis, in order to identify key pollutants causing the high toxicity of sediments. Results indicated different sets of toxicants inducing genotoxicity and metabolic activation. Overall, genotoxicity could be mostly related to alkanes, polychlorinated biphenyls (PCBs), naphthalenes (PCNs) as well as medium polar to polar polycyclic aromatic compounds (PACs), whereas AhR-mediated toxicity could mostly be attributed to polycyclic aromatic hydrocarbons (PAHs). A comprehensive evaluation of the ecotoxicological situation of sediments requires different approaches and broad knowledge for interpreting results. As exemplified for the Tietê River basin, a battery of bioassays applied in combination with chemical analyses and effect-directed analysis represent suitable tools to function as early warning systems not only for sediment pollution, but also for hazards for the entire river system.

TU 151

Acute sediment toxicity assessment in the river Sao Francisco, Brazil

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The São Francisco River is the fourth longest river from South America and the longest that runs entirely in Brazil. Although is a very important river, there are diverse anthropogenic activities, such hydroelectric plants, metal manufacturing industry or agriculture that have impact on these ecosystem. The objective of this study is to evaluate

by means of chemical and ecotoxicological approach the toxicity of the sediments of this river, and their elutriates, in a region downstream Três Marias reservoir, in a site near a processing industrial plant of zinc. For this, a battery of three laboratory bioassays, using two cladoceran (Daphnia similis and Cerodaphnia dubia) and one amphipod (Hyalella azteca) species, was used to assess the toxicity. 12 different metals (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and the different geochemical sediment fractions were determined a means of assessing the potential impact of the bioavailable fractions (two first steps of the sequential extraction procedure) in order to establish the potential effect of the industrial plant. The effect of sediment metal contamination was assessed applying multivariate techniques.
The results show elevated concentrations of Zn, Cd and Pb that involve an important potential risk on the aquatic environment. The sediments affected by the industrial plant presented high levels of toxicity, although the hydrodynamic of the river plays an important role in the metal distribution and consequently in the toxicity of the sediments.

TU 152
Ecotoxicological assessment of sediments from rivers impacted by a petroleum refinery
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Ecotoxicological studies are important for evaluating the effects and mechanisms of action of toxic agents in different environments. Aquatic sediments have a high potential for accumulating pollutants. As pollutants may become available in certain environmental conditions, the sediments can also become a potential source of contamination to water bodies. Thus, the assessment of sediment quality is essential to understanding the processes governing the fate and availability of pollutants in water bodies. For an efficient sediment ecotoxicology assessment, different approaches are necessary. The aim of the present study is to evaluate the ecotoxicological potential of sediment samples from three rivers impacted by a petroleum refinary, by means of Neutral Red assay (cytotoxicity), EROD assay (CYP1A induction potential), the sediment contact with fish embryos (acute toxicity and teratogenicity) and chemical analyses, among others. Since water samples from these rivers have being also investigated, these results must be associated, in order to obtain a comprehensive evaluation of the ecotoxicological situation of these water bodies. First results will be presented.

TU 153
Seasonal variation of toxicity and genotoxicity of sediments samples of the Ensenada de la Paz, B.C.S. México
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In this paper was carried out a qualitative assessment of toxic and genotoxic effects of sediment collected at 8 locations in the Ensenada de la Paz, B.C.S., in 2 contrasting seasons summer and winter of 2010, using a battery of biological tests with organisms of different trophic levels and the SOS-Chromotest microbioassay. Compounds with toxic and genotoxic effects were detected in 5 locations, located in the Canal de la Paz, near the termoelectric power station and the Pichilingue navy. LC50 and genotoxicity values observed, indicating the presence of toxic compounds at sites near Punta Prieta and Cicimar, sediment toxicity decreases with increasing distance from these points. This fact possibly indicates a contribution of harmful compounds probably from Punta Prieta and La Paz city to the Bay of La Paz. This type of analysis is essential to identify areas of risk in studies of environmental analysis.

TU 154
Toxic effects of sediments from a semi-arid coastal system, influenced by a gold ore mine
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More than 600,000 tonnes of pure arsenolite are scattered in the area of the El Triunfo gold ore mine, in the southern most Baja California peninsula, México. The drainage basin, from which sediments were collected for this study, discharges into the Pacific Ocean, however, the river is dry most of the year, only occasionally filled during tropical hurricanes in summer. This coastal system receives a continuous contribution of toxic compounds due to mineral extraction in the zone.
The aim of this study was to evaluate the toxicity of the sediments and the level of metal pollutants in order to determine the environmental risks. Heavy metals were determined in conjunction with sediment toxicity using Daphnia magna, Selenastrum capricornatum and seed-germination bioassay analysis. The Sediment Quality Triad (SQT) was used to identify areas in the system where sediment contamination may be linked to ecosystem degradation. A multivariate analysis was carried out and indicated that the degree of pollution varied from moderate to highly polluted. The analyses for the SQT indicate that the stations nearest to the alteration zone were severely polluted. These results suggest that there are health risks to human populations and activities such as vegetable and cattle farming or rural touristic recreation in the area. In order to restore the alteration zone to healthy conditions a remediation program would be necessary.

TU 155
Bioassays in sediment assessment for investigative monitoring in the context of the WFD
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An intense industrial activity has been taking place in Wallonia (Belgium) since the Middles Ages. The proximity to major waterways has enhanced the industrial development and has facilitated pollution toward aquatic environment. Sediments are known for their ability to trap pollutants and release these substances years or even decades after pollution has ended. In order to assess the current status of sediment pollution, bioassays can be used as suitable monitoring tools to answer the Water Framework Directive (WFD) requests and ascertain the causes of a water body failing to achieve the environmental objectives. They integrate all contaminants effects including additive and synergistic effects of unknown substances and breakdown products.
A sediment quality recurrent monitoring throughout the Walloon Region has been set up, taking into consideration physico-chemical parameters (including Prioritary List substances from the WFD) and ecotoxicological effects of collected environmental samples in a triad-type approach. Different species (Chironomus riparius, Heterocypris incongruens for whole samples, Vibrio fischeri, Pseudokirchneriella subcapitata and Brachionus calyciflorus for pore waters) are used in this approach because they provide different information due to the way of exposure (ingestion, tegumentary contact) and because they represent different trophic levels (decomposer, producer or consumer). Among the sediments of 10 stations studied in 2010, four showed a relatively low toxicity toward several organisms of the bioassays battery (the Wiltz, the Sure, the Gueule and the Rhosnes), 3 showed low toxicity toward one of the organisms (the Oise,the Molignée and the Eau d'Heure) and 3 showed no signs of toxicity (the Biesmes the Masblette and Salm) even if 2 non-toxics stations exceed the maximum acceptable levels as defined by decree of the Walloon government.
Results of the 2011 monitoring program will also be presented.

TU 156
Ecotoxicological assessment of contaminated rivers as a proxy for the water framework directive: an acid mine drainage example
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Metal contamination of freshwater water bodies resulting from mining activities or deactivated mines is a common environmental problem in Portugal. Despite authorities recognize its broad dimension with a variety of health-related hazards and threats to the environment, the establishment of an adequate remediation plan for each mining area is still failing or being too delayed. Braçal and Palhal, located in Central-North of Portugal in a riverside position, are both examples of deactivated mining areas lacking for recovery plans since their shut-down in the early-mid 1900's. Both mines are inserted in large underground complexes. Braçal was mainly focused on the extraction of galena ore and Palhal on the extraction of pyrrhotite, chalcopyrite, galena, sphalerite and pyrite ore. Since their shutting down, the mining run-off are running into two subsidiaries of Vouga River. The purpose of this work was evaluating the potential risks and hazards of the contribution of mining run-off into Vouga river subsidiaries for freshwater communities and nearby population villages. Therefore, ecotoxicological tests using a battery of assays were performed on sediments collected from the river in the vicinity of the mining run-off. The results shows that elutriates obtained from the sediments collected in Palhal mining area were toxic to all tested species, including the bacteria Vibrium fischeri, the algae Pseudokirchneriella subcapitata, the macrophyte Lemna minor and the cladocerans Daphnia magna and Daphnia longispina. In contrast, elutriates obtained from the sediments collected in Braçal mining area showed, in general, no toxicity for the several species, with only slight toxicity noticed to P. subcapitata. These results might be explained by the flooding of the Braçal mine that occurred following the mining shut-down, which slower the process of acid mine drainage phenomenon resulting from the oxygen in contact with ore. In opposite, Palhal mine never faced flooding events, which may explain the higher toxicity of the samples collected there. Our study highlights the usefulness of using an ecotoxicological approach to help the prioritization/scoring of the most critical areas impacted by deactivated mines.

TU 157
Assessment of effects of agro-chemicals use in ecological status of Kilombero Flood Plain, Ramsar site
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Tanzania is rich in wetland resources with an estimated 10% of the land surface covered by significant freshwater wetlands providing essential ecosystem and livelihood support functions. Millions of people depend on wetlands (MNRT, 2003) for fishing, agriculture, livestock keeping and collection of a multitude of minor wetland products, thus destroying the ecological functions of wetlands. Tanzania ratified to the Ramsar Convention on 2002, as a commitment to maintain the ecological character of its wetlands of international importance and to plan for the sustainable use of all other wetlands in the country. The Kilombero Valley, designated in the same year, is among four Ramsar Sites in Tanzania. It covers surface of 7,967 km^2 with a catchments area of about 40,000 km^2. Kilombero Valley is rich in endemic species of flora and fauna and characterised by high concentrations of large mammals especially Puku, Buffalo, Elephant, Hippopotamus and Lion.
Over the last two decades human settlement in the valley has increased, parallel with the socio-economic activities such as livestock keeping, fishing, cultivation, and bee keeping. The agro-pastoralism, large and small scale cultivation of rice and sugar cane with an intensive application of diverse arrays of pesticides, is a major threat to this valley´s ecological integrity. However, little is known about sediment chemical contamination and pesticide load of Kilombero wetlands. This is the first study to investigate the potential impact of toxic chemicals, especially agrochemicals, on the sediment-water quality of this flood plain. During a first survey, screening of the region for hot spots will be done by applying selected biotests that are sensitive to pesticides contaminated sediment and water samples. In a second tier, chemical analysis of those samples identified as hot spots will be carried out in order to identify sources. Water and sediment samples will be collected in both dry and wet seasons, the first survey being conducted between February and April 2012. Based on data from this first survey, a conceptual site model will be set up and presented.

The project's further objectives comprise the assessment of human and environmental risk due to pesticide exposure, the communication of these risks to farmers and inhabitants living in the area and development of management recommendations for a more efficient and less environmentally adverse use of this Ramsar site.

TU 158

Heavy metal pollution assessment in sediments of the Nemrut Bay, Turkey

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Surface sediments in the fraction <63 µm collected from eight stations in Nemrut Bay, Aegean Sea were analyzed for trace metals (Cd, Pb, Cr, Cu, and Zn) by using inductively coupled plasma atomic emission spectrometry (ICP-AES). Heavy metal concentrations in surface sediment varied from 0,1-0,95 mg/kg for Cd, 15-190 mg/kg for Pb, 13,8-58,65 mg/kg for Cr, 20,2-71,9 mg/kg for Cu and 8,09-178,3 mg/kg for Zn. The results show that some elements such as Cd, Pb and Cu are at risk level in the sediments near the harbour, harbour activity and petrochemical industry may be the sources of these elements.

TU 159

Determination of ecotoxicological effects of persistent organic pollutants from sediment cores of the Baltic Sea: a combination of chemical measurements and aquatic toxicity testing

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In the last recent decades persistent organic pollutants (POPs) gained increasing importance in environmental sciences and toxicology due to their carcinogenic and mutagenic effects to a diversity of organisms as well as negative impacts on human health. Attributable to atmospheric deposition and municipal as well as industrial waste water discharge POPs are distributed in the marine environment, where they are finally enriched in the sediments by the affinity to bind on particulate matter and sorption processes. Adverse impacts on the benthic fauna are caused through chemical uptake and bioaccumulation of these contaminants.
Ecologically relevant processes such as bioconcentration and baseline toxicity of POPs are mostly controlled by the chemical activity of the substance rather than by its total concentration in the sediment. To calculate chemical activity and thus to predict bioconcentration and the baseline toxic potential of a contaminant mixture the freely dissolved concentrations of PAHs and PCBs were measured in sediment pore water samples of the Baltic Sea using solid phase microextraction, a passive sampling technique based on the principle of equilibrium partitioning.
To evaluate biological effects of contaminated sediments, modern strategies not only focus on analyses of contaminants, but also test the impact on biological organisms. In addition to the chemical studies a composition of limnic and marine microbiological assays was used in order to assess bioavailability of the contaminated material to different organisms under controlled laboratory conditions. Interpretation of our biotest results followed a weight of evidence approach by which several individual positive tests in the tier add to an overall conclusion that a risk for the environment may in fact exist. The test system proved suitable for assessing the risk of the Baltic Sea sediments.

TU 160

Triad approach for risk assessment of sediment and floodplain soil of a metal-polluted stream

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Many streams and small rivers in Europe are faced with historic metal pollution due to the activity of zinc smelters. Rehabilitation activities are executed for many streams in the Netherlands, both in the sediment phase as in the terrestrial part of the floodplain. Especially the floodplains are faced with drastically changing environmental conditions due to the periodic and unpredictable flooding. During high discharge events, sediment is resuspended, transported, and settled at floodplains downstream. This spatial distribution of contaminants in the floodplain should be taken into account when streams and rivers are being reconstructed.
This is investigated in the Tungelroyse beek, a stream in the south of the Netherlands. Local water authorities have restructured large parts of the stream. We used a triad approach to assess whether all the ecotoxicological risks have been adequately removed by the restructuring and rehabilitation activities. Sediment and soil was sampled in the stream and in the floodplain on 5 locations along the stream. Total concentration (aqua regia extraction) and bioavailable concentration (CaCl2 extraction) of the following metals were analyzed: Cd, Cu, Pb, Zn, As. A standard Chironomus survival bioassay was conducted in the laboratory. Metal and bioassay results were compared with field inventarisations of the macroinvertebrate community.
The highest bioavailable concentrations of Cd and Zn were observed in the floodplain soils. The bioassay showed a negative effect for two locations. Combining the two measurements with the in situ macroinvertebrate community showed an ecotoxicological risk for one floodplain soil when inundated. These results are compared with previous studies along the river Dommel. Advices on optimal restructuring and rehabilitation of small streams should include the spatial distribution and bioavailability of contaminants.

TU 161

Hexachlorobenzene-contamination of sediments of the Upper Rhine River - identification of sorbents to assess bioavailability

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Due to historical emissions of hexachlorobenzene (HCB), the sediments of the ponds of Upper Rhine River in southern Germany remain highly contaminated (up to 1000 µg kg^{-1}). HCB is a bioaccumulative and toxic persistent organic pollutant (POP). By reason of its strong sorptive characteristics, HCB is mainly bound to organic substances. However, geogenic clay minerals are also capable sorbents. Regarding the contaminated sediments, the spatial distribution of HCB is considerably more heterogenic, then e. g. the distribution of polychlorinated biphenyls (PCBs). Further, maximum loads of HCB occur rather in larger particle fractions (63 - 2000 µm), than in smaller fractions (< 20 µm). Knowledge of the specific HCB-sorbents in the different fractions is of importance, because their characteristics control sorption strength, which is an important factor for remobilization as well as bioavailability of organic contaminants.
The mineral composition of sediments (measured by X-ray Diffraction, XRD) and its influence on the amount of sorption is presented, as well as composition and sorption capacity of sediment fractions (classified by particle size and content of organic matter). Sorption isotherms and partition coefficients (K_d and K_{OC}) were determined in batch equilibrium studies by solid-phase microextraction (SPME) coupled to gas chromatography (GC). In addition, adsorbents were investigated by imaging mass spectrometry and scanning electron microscope coupled to energy-dispersive X-ray spectroscopy (SEM-EDS).
Results contribute to explain mechanisms of sorption and can therefore help to predict remobilization processes as well as bioavailability of HCB in aqueous ecosystems. Results yield important physicochemical properties of sorbents for better understanding the environmental behavior and pathways of chemicals and beyond that to model environmental fate under changing climate conditions.
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TU 162

River sediment sampling and environmental quality standards

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Sediment forms an important part of the aquatic environment and provide habitat to benthic organism. However, poor water quality has been linked to contaminated sediment most of which consists of complex materials transported within river or deposited on river bed. Sediment has been identified as major transporter and sequester of fluvial contaminants such as heavy metals, most especially the fine sediment. At present, there are no environmental quality standards (EQSs) for bed sediments or fluvial suspended sediments, and one of the major problems in establishing sediment EQSs is the identification of the suitable sediment fraction to measure - the actively transported or previously deposited sediment. The variability in sediment characteristics is mainly attributed to their active properties and the characteristics of the rivers such as pH, redox, and high water discharge, which is investigated during sediment sampling.
The study aims to investigate the effect on particle size on heavy metal concentration, study seasonal variation in the concentration of Cu, Ni, Pb and Zn in Ravensbourne River, and compare the concentration of selected heavy metals between different compartments such as suspended sediment, bed sediment, bank sediment and the water column of Ravensbourne River. Sediment (bed and bank) and water samples were collected from Ravensbourne River over a period of 12months. Sediment samples were tested for heavy metal concentration using the aqua regia, sequential extraction using a 6hour extraction technique proposed by Maiz et al. (1997).

TU 163

The first sight into contamination of Polychlorinated biphenyls and Polybrominated diphenyl ethers in sediments of Tam Giang-Cau Hai Lagoon, Central Vietnam

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Rapid economic development and urbanization growth in Vietnam have made this country become an important subject for extensive studied dealing with environmental pollution recently. So far, there has been no information on PBDEs contamination in Central Vietnam and the information about PCBs sources and levels is limited. Tam Giang-Cau Hai Lagoon lies along the coastal line of Thua Thien-Hue Province, Vietnam. Due to recent industrial and economical growth in this region, large amount of untreated municipal and industrial wastewater have been discharged to this river - lagoon system, which may cause adverse environmental consequences and increased health risk for local communities. In this study, the contamination status, distribution and congener profiles of PCBs and PBDEs in surficial sediment of Tam Giang-Cau Hai Lagoon were investigated. PCBs and PBDEs were detected in all sediment samples with mean concentrations of 0.19 and 0.56 ng/g dw, respectively. The highest concentrations of both contaminants were observed downstream at the river mouths, indicating the river flow as a main source of pollutants into the lagoon system. The similarity of PCB congener distributions in sediments suggest the presence of a unique source over the entire study area, probably connected to local use of old electrical equipments containing

low chlorinated PCB formulations. Atmospheric transport & post depositional processes might modify to some some degree the fingerprint of PCB inputs to the environment favouring the predominance of 3, 4 & 5 chlorinated congeners. The PBDEs profile in sediment was relatively uniform, with decaBDE as the dominant congeners, followed by nona- and octaBDEs, indicating DecaBDE as the major technical mixture in consumer products in this region. In general, the levels of PCBs and PBDEs in lagoon sediments were low, compared with those reported in sediments of other rivers in Southern Vietnam as well as in other river systems in developed countries. This may reflect a limited usage of PCBs and PBDEs in this region. Comparing to sediment quality guidelines currently used around the world, present PCB sediment concentrations of Tam Giang-Cau Hai Lagoon does not constitute a threat to environment. Nevertheless, the first time detection of PBDEs in the lagoon-river system might be an indicator of enhanced recent human pressure that suggests the need for a monitoring plan to prevent the possible dangerous worsening in the near future.

RA07P - Environmental problems of estuaries

TU 166

Distribution character and potential risk of PCBs and phenols in surface water from 22 tributaries and mainstream in middle reaches of Yangtze River

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47 surface water samples were collected from 22 tributaries and mainstream of the Yangtze River from Chongqing to Yichang. The contents of 28 PCB congeners and 15 phenolic compounds in surface water samples were measured using Varian CP3800/300 GC-MS/MS technique. PCB8, 28, 52, 118, phenol, o-cresol, 2.6-dichlorophenol, and 2-nitrophenol are the most predominant compounds in the samples from tributaries, PCB8, 28, phenol, o-cresol, and 2-nitrophenol are the most dominant compounds in those from mainstream. The measured level of PCBs in the samples from the tributaries and the mainstream were 20.71 ng-L-1 and 13.25 ng-L-1, and the measured level of phenolic compounds in those samples from the tributaries and the mainstream were 87.99 ng-L-1 and 52.47 ng-L-1, respectively. The highest concentrations of PCBs and phenolic compounds were detected separately in the samples from Rangdu River (61.79 ng-L-1) and Jialing River (1723.77 ng-L-1). The average concentrations of PCBs and phenolic compounds in the samples are far lower than the Chinese drinking water guideline values. The concentrations of PCBs and phenolic compounds in the samples were in the same order of magnitude of those reported on lower levels in European and American countries, which show that potential risk are negligible due to PCBs and phenolic compounds contamination in these samples.

TU 167

Measuring trace labile metals in the Pasig River water using DGT technique: an application for an effective water resource management

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Pasig River is an important water body in Manila, Philippines. This is a busy river greatly influenced by urbanization. It is an estuary in nature as it is in between a bay (Manila Bay: west) and lake (Laguna Lake: east). The water quality is a main concern. From being in a state of biologically dead, rehabilitation is applied to raise it to Class C. The efforts are still on-going. An efficient water quality monitoring technique and assessments are necessary. This research made use of the current available technique called passive sampling. DGT had been used as the target compounds are the labile trace metals (Cr, Co, Ni, Cu, Zn, Cd and Pb). The time of sampling campaigns captured the ENSO phenomenon (El Niño: Period 1, La Niña: Period 2 and in between summer and rainy seasons: Period 3). From these periods, DGT-labile metal concentrations had been computed. Multivariate analyses composing of the Principal Component Analysis (PCA) and Factor Analysis (FA) were employed. This is to be able to identify the parameters that play a part more in each period. The cumulative percentages of the total variance are as follow: 89.3% for Period 1; 87.11% for Period 2; and 81.25% for Period 3. Correlations among the parameters had been achieved as well. In addition, percent concentrations of each element per site were determined. Scores were given and ranking was then supplied. Based from the ranking, sites that contribute more of the selected parameters were identified. For Periods 1 and 2, highest contributions are found near mid to upstream. While for Period 3, highest rank is near at the downstream. From this also, we were able to locate the sites that can be given more priority and attention. Fluxes (in kg d-1) were provided as well. Period 2 having the highest flow gave the highest fluxes per site.

TU 168

Dioxin-like compounds in Portuguese estuaries: levels and patterns in superficial sediments

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Persistent organic contaminants such as polychlorinated dibenzo-p-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and dioxin-like polychlorinated biphenyl (DL-PCBs) may enter the estuarine system through atmospheric deposition, riverine runoff and direct release from human activities. In the aquatic environment these contaminants strongly adsorb to suspended particles and tend to accumulate in underlying sediments which constitute long-term reservoirs and secondary sources. Contaminated sediments can therefore represent a significant risk to aquatic life and human health and the loss of natural and economic resources. The aim of this research is to evaluate the sediment contamination of Portuguese estuaries by PCDD/Fs and DL-PCB. Seven estuarine systems were selected along the Portuguese coast: Lima, Ria de Aveiro, Mondego, Tagus, Sado, Mira and Ria Formosa. At each estuary, superficial sediments (0-10 cm) were collected at various locations in the intertidal mudflats. Samples were analysed by high-resolution gas chromatograph/high-resolution mass spectrometer (HRGC/HRMS) to determine the concentration of seventeen PCDD/Fs and twelve DL-PCBs. Results show that PCDD/Fs and DL-PCBs concentrations are variable not only between estuaries but also within each estuary. In general, the highest values were found at sites near large populations and industrial complexes (e.g. Tagus, Ria de aveiro and Sado). The lowest PCDD/Fs and DL-PCBs values were measured in Ria Formosa, a protected area with status of Nature Park. OCDD is the most abundant PCDD/F congener in all the samples but PeCDD or PeCDF were the major contributors to WHO-PCDD/F-TEQ2005. For DL-PCBs, PCB 118 was the dominant congener while PCB 126 was the major contributor to WHO-PCB-TEQ2005 in all the analysed sediments.

TU 169

Environmental monitoring and assessment of Terminos Lagoon, Mexico

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Terminos lagoon is located in the estuary of the Grijalva-Usumacinta river system, with the largest fresh water runoff in Mexico (35% of the total), and the second largest in the Gulf of Mexico. It is near of one of the main oil drilling areas in Mexico and there are many industrial facilities associated with the oil industry affecting the region. Other industrial activities, such as urban waste water, and agriculture, are also important inputs of pollutants to the Gulf. All these activities introduce pollutants such as metals, hydrocarbons, pesticides, etc.

As one of the components of the Sustainable Management of the Gulf of Mexico Large Marine Ecosystem, the pilot project on monitoring and environmental assessment selected Terminos as the site to study. The project consists of five modules: water quality, sediment quality, contaminants and biomarkers in fish, benthic community, and habitat degradation. The results are categorized as good, regular or bad according to pre-established criteria, in some cases adjusted to conditions in the southern Gulf. Sampling was random stratified, using the five zones determined from hydrology as a basis for stratification. Results show that in general pollutant concentrations in sediments are low, and have been decreasing at least since 2005. However, mercury levels exceed Mexican advisory levels in the muscle of fish in about 20 % of the fish. For organic pollutants there were no significant differences between the zones, except for HCHs and PAHs. A number of stations were found to be in "bad"condition for water quality and benthic community. Loss of mangroves forests is decreasing. Results have been presented to management authorities.

TU 170

Chemical and biological assessment of metal pollution in the Urdaibai Reserve of the Biosphere (UNESCO) using oysters as sentinels

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Oysters have been widely used as sentinel organisms in order to determine the presence of pollutants and their effects in coastal and estuarine environments. In the present work, oysters from the Oka river estuary (Urdaibai Reserve of the Biosphere, UNESCO) were collected in 7 sites at different seasons along 2010. Together with oysters, water and sediment samples were also collected. The concentration of 14 elements (Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn) was determined. Cell and tissue level biomarkers and histopathological alterations were also recorded. High Cu concentrations were present in the water column but tissue concentration was not high in oysters. Overall, some seasonal trend was observed in metal concentration in the water column with higher values in September, which was not reflected in oyster tissues. In contrast, a moderately high Pb concentration was found in oysters but not in water. Preliminary data in sediments indicated higher values of Cu and Mg in Mundaka. Regarding cell and tissue level biomarkers, no significant differences were observed comparing the sites. Occasionally, Arteaga presented symptoms of atrophy in the digestive diverticula in March and June. Seasonal variability was observed in the Connective-to-Diverticula ratio, with lowest values in September. Likewise, seasonal changes in the cellular distribution of metals as also observed in the digestive gland. Metals revealed by autometallography as black silver deposits (BSDs) appeared in the basal lamina (histological sense) of the digestive epithelium in March and June, whereas BSDs were localized within digestive cell lysosomes in September and especially in November,. The when a significant increase in the BSD volume density was observed in parallel with an increased metallothionein content. In conclusion, the results confirmed that the Urdaibai Reserve of the Biosphere presents low-to-moderate levels of metals both in oysters and waters, with only minor biological effects in oysters. It is worth noting that, however, seasonality may play a relevant role in environmental metal levels, bioaccumulation and the biological responses of oysters against environmental insult.
Acknowledgements: This work has been financially supported by the UNESCO Chair of the UPV/EHU through the UNESCO 09/23 project and by the UPV/EHU trough the Consolidated Research Groups (Ref.: GIC07/26-IT-393-07).

TU 171

Monitoring of sediments genotoxicity of the Yucateco, lagoon Tab, México

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The Yucateco lagoon is located in southeastern of the Mexican Republic in the Tabasco state. This system receives a continuous contribution of toxic compounds due to hydrocarbons extraction and industrial activity carried out in the zone. Since the system have been reported mass mortality events of fish and shellfish, in this research, an evaluation of the genotoxic effects of sediments over a period of 5 years were carried out to establish the state of ecosystem health. Sampling were made annually during the dry and rainy seasons. porewater were obtained from sediment samples, which were tested in the SOS-Chromotest microbioassay. In general can mention that the number of sediment samples with genotoxic effects decreased from 2003 to 2007. In 2003 was detected genotoxic compounds in 5 sampling stations, while in 2007 this effect was observed only in 3 locations. Critical areas are the stations 1, 2 and 3 located in the northern and central part of the system, the genotoxic effects decreases with increasing distance from these points in the direction of outlet the river Tonala. The critical pollutants likely to cause genotoxicity were the HAP's, PCB's (polychlorinated biphenyls) and metals vanadium and nickel. The utility of this type of analysis is essential to identify areas of risk in studies of environmental analysis.

TU 172

Bang for buck: the challenge of choosing environmental indicators of anthropogenic impacts in estuaries

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Ecological assessments over large spatial scales require that anthropogenic impacts be distinguishable above natural variation, and that the monitoring tools implemented maximise impact detection for a relatively low cost. For three heavily modified and four relatively 'pristine' estuaries (disturbance category), chemical indicators (metals and PAHs) of anthropogenic stress were measured in benthic sediments, suspended sediments and deployed oysters, together with other environmental variables. The differing ability of these data sets to explain the variances observed in infaunal and hard-substrate invertebrate communities were compared. Univariate analyses were useful for comparing contaminant loads between different monitoring tools and identified the strongest correlations between benthic and suspended sediments. However, multivariate analyses were necessary to distinguish ecological response to anthropogenic stressors from environmental "noise"over a large spatial scale and to identify sites that were being impacted by contaminants. These analyses provide evidence that suspended sediments are a useful alternative monitoring tool to detect potential anthropogenic impacts on benthic organisms.

TU 173

Metal availability and toxicity in estuarine systems in relation to chemical speciation and biological diversity

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The estuarine environment is a very dynamic system characterized by strong physical and chemical gradients and an enormous diversity in flora and fauna which undergoes major transitions moving from the freshwater to the marine areas. Along these gradients metal concentrations and speciation undergo important changes which also have an impact on the environmental distribution and biological availability of the metals.Parallel to this are changes in the physiological organisation of the biological species living in this gradient as they adjust themselves to the changes in ion composition and osmotic pressure of the external environment in order to maintain homeostatic balance.
The effect of changes in salinity on metal speciation, metal biouptake and metal toxixity was studied under controlled conditions in a series of structurally and functionally different organisms with either a freshwater or marine origin (e.g. unicellular algae, water flea, blue mussel, periwinkle, sea bass,...). The results were compared and combined with information available from data bases and literature to explore whether general concepts such as the free metal ion activity model and major ion competition effects used to describe metal uptake and toxicity remain valid across estuarine gradients and biodiversity. Using a dynamic modelling approach the effects of changes in physical and chemical conditions on metal speciation, biouptake and toxicological effects have been analysed and simulated across species diversity. For some metals, such as cadmium; where a strong inorganic metal speciation effect is observed a species sensitivity distribution analysis shows a decrease in metal toxicity moving from freshwater to higher salinity environments. However, this is not the case for a metal such as copper and in any case the effect is considerably less that what would be expected on the basis of the changes in free metal ion activity. The results of these observations are further discussed in relation to the ion and osmoregulatory constraints of the different species and whether or not normalization of metal availability and toxicity on a free metal ion activity scale is warranted for risk assessment purposes in estuarine gradients.

TU 174

Ecological risk management at a site adjacent to an area of special scientific interest

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Risk assessment is currently underway for a contaminated site in Northern Ireland with a wide variety of compounds of potential concern. In accordance with the UK CLR11 framework for management of contaminated sites, risk assessment is integral in leading the site from investigation to remediation, with the aim of protecting human health and the environment and obtaining a "suitable for intended use"outcome. An important feature of this site is its close proximity to an Area of Special Scientific Interest (ASSI), a Special Protection Area (SPA) and a Ramsar site. Being adjacent to such sensitive receptors, the ecological risk management approach for this site is a high priority.
This poster presentation aims to share some of the recent experiences and future directions in ecological risk management at the site, including:
- Ecosystems are complex, therefore identifying and monitoring the right parameters is key to successful management. At this site, environmental monitoring has extended beyond routine water quality parameters to include terrestrial and aquatic ecological surveys and detailed studies evaluating benthic community abundance and diversity. These will form a robust baseline for evaluating changes in ecological status as short- and long-term remedial measures are implemented.
- Early engagement with regulatory authorities is important to agree benchmarks for assessing environmental monitoring data. Being able to understand and critically review how these benchmarks are established is key in effective engagement on these issues. Identification of a subset of key risk-driving compounds has helped facilitate assessment of potential impacts from multiple sources/ stressors.
- Stakeholder engagement beyond the regulatory authorities is important to communicate the management approach and seek alignment with social as well as scientific drivers. Using sustainability indicators to support remedial decision making on the site can assist with more fully integrating ecological risk management into the process.
- Ecosystems are constantly changing, therefore it is important to continually review the monitoring and assessment approach and consider the influence of potential future stressors, such as climate change.

TU 175

Integrated estuarine Mmdeling to support watershed management for sinclair and dyes inlets, Puget Sound, WA, USA

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An integrated estuarine model was developed to simulate fecal coliform (FC) fate and transport in Sinclair and Dyes Inlets, Puget Sound, WA. The integrated model consisted of a watershed model (Hydrologic Simulation Program Fortran - HSPF), an empirical fecal coliform loading model based on upstream land use and cover (LULC), and three dimensional fate and transport model, Curvilinear Hydrodynamics in Three Dimensions (CH3D), previously calibrated to match the hydrodynamics of the Inlets and modified to include FC kinetics (CH3D-FC). The integrated model was used to simulate the release, transport, and fate of FC loading from watershed pour points corresponding to stream mouths, stormwater outfalls, waste water treatment plant discharges, and shoreline drainage areas. A total of 20 simulation scenarios were run to verify model performance, assess sensitivity and uncertainty, and provide results needed to establish management goals for the watershed. Model results indicated that for marine waters to meet standards, FC targets more stringent than the freshwater quality standards were needed for three streams that discharged in nearshore areas with reduced flushing. Overall, the integrated watershed-receiving water model performed very well. The integrated model was able to recreate a wide range of dynamic loading within the inlets, from large-scale storm events with high flow conditions to dry, low-flow conditions during the summer months. The integrated watershed monitoring and modeling approach to water quality management is assisting the development of management plans worthy of stakeholder acceptance, helping to achieve reductions in FC loading, and resulting in improvements to the environmental quality of the inlets.

TU 176

Impacts of hypersaline conditions on the endocrine and reproductive effects of bifenthrin on salmonids

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Pyrethroids are a commonly used class of pesticides in California in both agricultural and urban applications. Although pyrethroids are reported as having nominal effects in mammals, they have been shown to have toxic effects in aquatic organisms, especially invertebrates and fish. Additionally, studies have found that in parts of the San Francisco Bay Estuary system, salinity has been increasing as much as 3 parts per thousand (ppt) each year. Such increases in salinity have been shown to increase enzymes that may reduce the acute toxicity of pyrethroids, but may form metabolites that have greater endocrine disrupting (i.e., estrogenic) activity. Concentrations of bifenthrin, a third generation pyrethroid, have been observed to be as high as 73 ng/L in northern California waterways. To evaluate the effects of hypersaline conditions on bifenthrin toxicity and endocrine disrupting activity, sexually immature rainbow trout (Oncorhynchus mykiss) were exposed for 14 days to nominal concentrations of 10 and 150 ng/L. Prior to bifenthrin exposure, fish were acclimated to freshwater, 8 and 17 ppt saline conditions. As hypothesized, 85.6 % mortality was observed in the 150 ng/L treatment

group in freshwater fish, 14.4% in the 8 ppt and no mortality in the 17 ppt salinity treatment groups indicating protection by hypersaline conditions from acute lethality. However, significant differences were not observed in plasma vitellogenin from bifenthrin-treated fish acclimated to fresh and salt water. These data suggest that higher salinity environments enhance the detoxification of bifenthrin, but without enhancing endocrine disrupting activities at these concentrations for 14 days.

TU 177

Characterization of organic and inorganic mercury transference dynamics in a coastal lagoon food web using stable isotopes

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It is well established now that, the determination of total Hg (HgTOT) concentration is not sufficient to understand its fate in the environment. Inorganic ionic Hg is the main form in waters and sediment samples, whereas organic forms (HgORG) account for the majority of Hg found in biological tissues. Despite not representing a large fraction of HgTOT in the aquatic environments, the main form of HgORG, methylmercury (MeHg+), raises prime concern, due to its tendency for bioaccumulation and biomagnification within the aquatic food chain. In order to examine the trophodynamics of HgTOT and HgORG in a coastal lagoon, suspended particulate matter (SPM), sediment, seston, phyto and zooplankton agglomerates, Crangon crangon, Pomatoschistus microps, Liza aurata, Atherina boyeri and Dicentrarchus labrax were sampled. Sampling took place near the mouth of the two major freshwater sources (Vouga and Antuã rivers) to the lagoon. Vouga River runs in a narrow channel, with steep margins. Its watershed receives influences from agriculture, livestock production, urban, industrial and mine effluents. Laranjo Bay (a depositional area) receives the Antuã, which has been conveying industrial discharges, particularly an effluent from a mercury cell chlor-alkali plant (from the 1950s until 1994). From the estimated 33t of mercury dispersed into the lagoon, the majority (81%) is associated with the sediments in Laranjo bay. By determining the position of each of the sampled compartments in the local food webs, using GC-C-IRMS (Gas Chromatography Combustion isotope Ratio Mass Spectrometry) to quantify stable isotopes ratios (δ13C and δ15N), the evaluation of this element's transference in the food web was achieved. Mercury analysis was executed using CV-AAS (Cold Vapor Atomic Absorption Spectrometry). Certified reference materials (DOLT-3 and DOLT-4 for tissues, PACS-2 for sediment) were used in the QC/QA process. A significant negative correlation (Pearson Product Moment correlation, $P<0.05$) and a very strong positive one (Pearson Product Moment correlation, $P<0.0001$) were found for Laranjo data, between HgTOT and δ15N, and HgTOT and HgORG, respectively. No other significant correlations were established.
Results for HgTOT were generally in line with published values for Laranjo Bay. Organic mercury was the most abundant of the species in the superior organisms (always>57%). The diverging patterns of HgTOT and HgORG distribution are discussed.

TU 178

Is chronic exposure to pollution able to change the physiological capability of Corbicula fluminea to respond to acute chemical stress in estuaries?

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The Asian clam Corbicula fluminea (Müller, 1774) is an invasive species that has been colonizing aquatic ecosystems in Europe, America and several other regions. In the freshwater tidal area of the Minho River estuary (NW Iberian Pensinsula) that was colonized by this species in the 80s, C. fluminea is now the dominant species of the benthic community, has been contributing to the decline of several native molluscs and considerably changed ecosystem functioning. Furthermore, C. fluminea has been showing to be able to develop resistance to pollution. Here, the hypothesis that individuals from the river but inhabiting sites with distinct levels of historical contamination, have different capabilities of answering to pollution exposure events. To test the hypothesis, animals collected at two sites in the Minho River under different anthropogenic pressure were exposed separately to the model polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP) after a period of acclimation to lab conditions. At the end of the bioassays, enzymes involved in neurotransmission, biotransformation, anti-oxidant defences, aerobic energy production and lipid peroxidation levels were used as biomarkers. Results show differences in biomarkers responses between the two groups of clams suggesting that animals inhabiting the most contaminated site developed tolerance to chemical stress.
This study was carried out in the scope of the project "NISTRACKS - Processes influencing the invasive behaviour of the non indigenous species Corbicula fluminea (Mollusca: Bivalvia) in estuaries - identification of genetic and environmental key factors"funded by the Portuguese Foundation for the Science and the Technology (FCT) (PTDC/AAC-AMB/102121/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). P. Vilares had a grant in the scope of the project and C. Oliveira had a PhD grant from FCT (SFRH/BD/68423/2010) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Tipology 4.2.).

RA19P - Plants and chemicals in the environment: risk assessment,pest management and phytoremediation

TU 181

Sediment contact test with Myriophyllum aquaticum (ISO/CD 16191): first results of an international ring test

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Although they are an important part of an aquatic ecosystem, dicotyledonous macrophytes are not yet part of the risk assessment of sediments or dredged material.
A sediment contact test system with the dicotyledonous watermilfoil Myriophyllum aquaticum was established by the ecotoxicological laboratory of the German Federal Institute of Hydrology. This test procedure is now in the standardisation process within ISO.
In the sediment contact test, Myriophyllum aquaticum whorls are exposed to environmental samples for ten days. The growth of Myriophyllum aquaticum in a test sample is compared with its growth in the control sample. Phytotoxic effects can be quantified as growth inhibition (%) relative to the control growth.
Several laboratories from academia, industries and government participated in an interlaboratory ring test organized by the German Federal Institute of Hydrology. The ring test aimed at i) investigating the practicability and reproducibility of the sediment contact test, ii) validating the chosen endpoint, and iii) determining the response range of the reference substance functioning as positive control.
First results of the interlaboratory ring test will be presented.
Acknowledgement: U.F on behalf of the participants of the international ring test

TU 182

Getting more ecologically relevant data from laboratory tests: recovery potential of Lemna minor

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The goal of ecological risk assessments for chemicals typically is to protect the status of populations. Still, the assessments are usually conducted at the level of the individual, so the results of laboratory testing need to be translated into predictions of population status. Extrapolation from laboratory tests has many limitations, among which is the fact that species differ not only in toxicological sensitivity but their potential for recovery from adverse effects.
Lemna minor and L. gibba are representatives of rare standardized laboratory test species that can actually provide direct information on population status upon exposure to potentially harmful substances. Still, the standardized laboratory protocols are typically completely ignoring the recovery potential after exposure. Such ecologically relevant information can be gained already from suitable minor adaptations of standard laboratory tests. In the study on sensibility and recovery potential after exposure to atrazine (Teodorovic et al, ETC, in press) we suggested that standard test protocols with Lemna sp. could be adapted by a) adding a recovery phase after the exposure phase and b) frequent observations and measurements (in 3 days long intervals) during the whole study.
The suitability of adapted protocol has been tested on three substances with different mode of action: standard reference substance for Lemna (3, 5 DCP in concentration range 0.625-10 mg/l), and two herbicides - isporoturon and trifluralin (in concentration range 0.01 - 1 mg/l). The results indicate that adding the 7 days long recovery phase after the exposure phase is suitable adaptation of the standard test protocol. Frequent observations and measurements during the whole study, particularly during the recovery phase, can provide a better understanding of response and recovery patterns. Relative growth rate calculated for the last 3 day long interval of the study (Last interval RGR) proved to be a suitable and sensitive end point which might provide good indication of plant recovery potential after exposure to toxic compounds. Estimation of recovery potential directly from suitable laboratory tests would add more ecological realism into worst case scenario responds typically obtained by single species laboratory toxicity test but also provide valuable data for population - level models.

TU 183

New developments in mesocosm testing with herbicides: recent experiences and recommendations

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A number of new developments in mesocosm testing have taken place since the most recent revisions to guidance for these complex studies (e.g. HARAP, 1998 & CLASSIC, 2001). Much of the progress in this area since this guidance was produced has been in the design and conduct of freshwater mesocosm studies to evaluate the toxicity of herbicides on algae, periphyton and macrophytes. This presentation will highlight some of the developments in this area using examples drawn from recent state of the art mesocosm studies.
We will highlight our recent experiences in this area by describing methods we have developed to evaluate the effects of herbicide exposure on a variety of macrophytes with different morphology and life histories. We will also provide recommendations for the evaluation of effects on algae and periphyton drawing on our experience in this area. It is hoped that the information provided will advance mesocosm study design and offer advice to those considering conducting mesocosm studies with herbicides whilst providing a catalyst for discussion in this developing area of higher tier aquatic ecotoxicology.

TU 184

Unforeseen effects of herbicides on wetland and terrestrial plant communities: time to vegetative and reproductive recovery following exposure

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Standard plant toxicity tests rely on the evaluation of species in short-term (ST) studies; long-term (LT) effects of low doses of herbicides on wild vegetation, or the recovery potential of plants is often not assessed. The ability of wild species to recover following sub-lethal exposure to herbicides was examined using the sulfonylurea herbicide chlorimuron ethyl. Eight wetland and nine upland species were tested with eight doses of chlorimuron (+ controls) ranging from 1 to 107% of the recommended label rate

of 9 g ha-1. For each species, two sets of plants were grown: total aboveground plant biomass was assessed at four weeks after exposure for the ST plants (first set) and again for the LT plants (second set) when the controls began natural senescence (7-23.5 weeks post-spray depending on species). To assess recovery over time, measurements of maximum plant height were taken weekly for all species using the LT plants. Reproductive parameters were also recorded for 13 species. Inhibition concentrations (IC50) were calculated for ST and LT biomass and for LT reproductive parameters. ST biomass was found to be the most sensitive measure of IC50 for nine species and LT reproduction for three; IC50s could not be predicted for five species (no effect). Delays in flowering time were apparent for several species. Twelve species had early delays in growth as compared to the controls at doses of 7.4% or less, two species were affected at higher doses (14.5 or 55%) while three species (all grasses) had no reductions in height at any dose. Of the species affected at doses of 7.4% or less, three fully recovered within an avg. 6.7 weeks post-spray, six recovered up to doses of 14.5% (avg. 6.5 weeks) and three up to doses of 28.2% (avg. 12.3 weeks). Though an overall trend towards recovery was also noted for reproductive parameters (4 spp. recovered, 3 remained the same, 3 unaffected at any dose), rates of recovery did not follow those observed for height. Delayed growth and flowering was observed at doses less than known drift rates (<10%); however, plants exposed to these low rates were often able to recover over time. While recovery did occur in this greenhouse experiment, it may be suppressed in natural communities where more resistant species (e.g. grasses) may grow faster and out-compete susceptible ones for light or resources. Such events may alter the natural species composition in exposed areas. This community approach is not considered in risk assessment.

TU 185

Effects of agrochemicals on macrophyte community structure and quality in a Canadian agricultural watershed

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Macrophytes are essential to healthy rivers and lakes. Unfortunately, macrophyte diversity, particularly diversity of submerged species, appears to have declined in some North American and European ecosystems during the last century. In agricultural watersheds, streams are intimately connected with croplands and the health of macrophyte communities may be compromised by increased levels of disturbance and exposure to agrochemicals such as nutrients and herbicides in areas of high-intensity agriculture. The macrophyte community was identified at twenty-four sites located throughout the South Nation River watershed, a sparsely populated agricultural watershed comprising 3919 km^2 in Eastern Ontario, Canada. The sites varied in terms of their contamination with nitrate and the commonly used herbicide atrazine, with mean spring maximum in-stream concentrations of 6600 µg/L and 1.56 µg/L respectively. In-stream concentrations of nitrate and atrazine were strongly correlated. Over 200 macrophyte species were identified along stream banks and within the stream channels. Sites surrounded by high levels of agriculture tended to have a higher percentage of non-native species, suggesting that native species are losing ground across the watershed. A decline in the quality and conservation values of macrophyte communities was observed along a gradient of nitrate contamination.

TU 186

The effect of five sulfonyl-urea herbicides to aquatic macrophytes - implications of results from outdoor growth inhibition studies for the risk assessment

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The collection of multi-species data is an option to refine higher tier risk assessments. The objectives of multi-species testing are (1) to derive a species-sensitivity distribution (SSD) and (2) to identify a most sensitive species. Lemna is the standard aquatic test organism and among the most sensitive species to herbicides. Multiple species data provide arguments to lower the assessment factor due to increased realism and lower uncertainty. The example of sulfonyl-urea herbicides (SU) outlined here shows that herbicides with the same mode of action can have the same or a similar SSD for aquatic macrophytes.
Over a period of four years Smithers Viscient (sponsored by Bayer CropScience) generated macrophyte data for five different SU herbicides. In each study 9 to 10 species from a variety of taxonomic groups were exposed in artificial outdoor ponds for 6 to 8 weeks. Each year the design was slightly adjusted based on experiences gained from former tests. Despite these changes, the data obtained are applicable to (1) comparisons of species sensitivities within one test substance and (2) comparisons between the test substances.
Numbers of replicates were 4 in the controls and lowest concentrations, 3 at medium, and 2 at the highest concentrations (combined NOEC-ECx-design). A dose-response was observed in most species. Due to variation among replicates, the EC50 is regarded as more reliable than the NOEC. As plant length could not be assessed in all species, dry weight biomass was used for comparisons. EC50-levels based on biomass and biomass growth rates were very similar.
The EC50-levels from biomass growth rates were compared to the Lemna-EC50. In 3 of the 5 herbicides tested none of the macrophytes was more sensitive than Lemna. For 2 substances sensitivity of Lemna was ranked 2 and 3. In all 5 cases Lemna was among the 5% of the most sensitive species. Myriophyllum, the forthcoming second standard species within the frame of EU regulations, was of medium sensitivity (rank 4 to 6). Overall, the pattern of sensitivity distributions was very similar in all 5 SU herbicides.
The large amount of data obtained enables refined risk assessments with low assessment factors based on the lowest EC50 as well as probabilistic approaches using a hazard concentration (HC5).

TU 187

Ecological functions of plant growth regulators in the stability of agrocenosis

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Tolerance of the system “soil-plant”in agrocenosis is the key condition of high yield. These functions in soil are accomplished mainly by humus component, and in the plant - by complex of biologically active compounds. Regulatory role in the resistance and protection of agrocenosis is studied in the example of chlorcholinechloride (CCC) and phytohormones: 24-epibrassinolide (EpiBS), gibberellins (A3). Their functioning and efficiency in “soil - plant”system is connected with a series of abiotic (temperature, nutrient elements, etc.) and anthropogenic (organic pollutants, etc.) variable factors. The complexity of agro-chemical and ecological studies presupposes the widening of methodical approaches. The wide use of bio-testing methods for summary characteristics of agrocenosis functioning allowed the revealing of protective effects of CCC, EpiBS, A3 of the, which are widely implemented today into the practice of agriculture. The protective effect peculiarities of CCC were studied at a high load of agrocenosis by chemical means of plant protection. It with the reduction of total toxicity in soil, root system, in plant and reproductive organs were revealed during using CCC Correlation of biological activity by bioassay used in agricultural lands, was found, depending on the plant nutrition system. Therefore, foliar effect of CCC influences on the plant, changes the activity of root system functioning (growth, absorption and root excretion), that actively effects on the dynamics of microbial association. The important ecological role of exogenous use of EpiBS phytohormone was proved experimentally. The optimum created in agrocenosis artificially (providing plants with elements of mineral nutrition) is shifting under the influence of abiotic factors of external medium (temperature, humidity, light, etc.). EpiBS takes part in the regulation of plant adaptation by constantly changing the complexity of these factors, depending on each other. Use of EpiBS is compatible with gibberellins (A3). Efficiency from their joint use is manifested in personal contribution of each to the performance of definite functions in plant ontogenesis. Thus, the biologically active compounds directly and indirectly affect the stability of agrocenosis. Intensive implementation of plant growth regulators into the practice of agriculture is connected with their expressive, smoothing and systematic effect.

TU 188

Biochemical responses in the aquatic macrophyte Myriophyllum quitense exposed to the strobilurin fungicide azoxystrobin

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Strobilurin fungicides constitute a relatively new fungicide class developed from natural fungicidal derivatives and the great impact of them on agriculture is reflected by the widespread use of azoxystrobin (AZX), a compound approved for use on almost 100 different crops. Its mechanism of toxicity is well understood in fungi but it effects in plants is scarcely studied. Aquatic macrophytes have been used as environmental biomonitors because of its bioacumulation capacity of xenobiotics. The main goal of this study was to establish a concentration- response curve of AZX in *Myriophyllum quitense*, by using oxidative stress enzyme activities and chlorophyll content. Particularly, the activity of Guaiacol Peroxidase (POD), Glutathione- S -Transferase (GST), Catalase (CAT), and the chlorophyll a and b contents were tested. Plants were exposed to 0 (negative control); 0,1; 1; 10; 50 and 100 ug /L of AZX for 24 hours. All the parameters were measured espectrophotometrically. Inhibition of CAT and POD at 50 ug/L was observed ($p<0.05$), showing the stress condition exerted by this fungicide. GST activity was not significantly different to the control at any concentrations tested ($p>0.05$). Although there was no change in the chlorophyll a/chlorophyll b ratio among treatments an increased content of both pigments at 100 ug/L with respect to control was observed ($p<0.05$). Our results shows the importance of using biomarkers of different sensitivity, in the evaluation of potential negative effects of emergent agrochemicals in the environment, being a more detailed studied highly recommended.

TU 189

Impact on surface water, stream sediments and macrophytes from the Aljustrel mining area (Portugal)

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The main environmental problem in the vicinity of Aljustrel mining area, as in other massive sulphide deposits of the Iberian Pyrite Belt, comes from the tailings deposited at the site which contain large concentrations of trace elements (namely As, Cu, Pb and Zn) and can react with oxygen and water producing acid mine drainage (AMD). The AMD produced will end up reaching the downstream areas, affecting surface water, groundwater and stream sediments. That impact is evident at Água Forte stream, which receives the AMD from the Aljustrel mine area, a tributary of the Roxo stream.

The aim of the study was: a) to evaluate the extent of pollution in stream waters and sediments at Água Forte and at Roxo, and b) to assess the potential of the macrophyte *Scirpus holoschoenus*, collected along the stream banks, for phytoremediation of AMD. Several sampling sites were selected at Água Forte stream and at Roxo stream, upstream and downstream from the confluence of the Água Forte stream. Water quality was assessed considering physico-chemical characteristics and ecotoxicological parameters. Sediment samples were analyzed considering general physico-chemical characteristics and a sequential extraction procedure was used to quantitatively assess trace element pool (As, Cu, Pb and Zn). *Scirpus holoschoenus* samples were analyzed in order to assess trace element concentrations in the aboveground plant material.

Both surface water and sediments were extremely acidic at Água Forte stream, with pH values <2.92 for surface water and <3.32 for sediment samples. Surface water at Roxo stream presented pH values >7.26, apparently not affected by the confluence of the Água Forte stream, but the same was not true when considering the pH and other characteristics of the sediments. Ecotoxicological biossays (luminescence inhibition of *Vibrio fischeri* and 48-h immobilization/mortality assay with *Daphnia magna*) were consistent evidencing that water samples collected at the Água Forte stream were extremely toxic, with very EC50 values. Although high As, Cu, Pb and Zn concentrations were found at Água Forte stream, the same was not true in Roxo stream. Trace element content in water, sediments and *Scirpus holoschoenus* samples was analyzed and correlated in order to ascertain if this particular macrophyte, which seems adapted to the extreme conditions found at Água Forte stream, can be potentially used for metal removal in constructed wetlands to treat AMD.

TU 191

Species-specific responses to zinc in Lemnaceae: zinc storage and impacts on photosynthesis along frond developmental gradients

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Lemnaceae are used to represent all macrophytes in standardised aquatic toxicity testing, with protocols allowing for the use of different species. Recently, differential sensitivity in Lemnaceae species to zinc, based growth and cholorphyll-a fluorescence parameters, has been reported. Chlorophyll-a fluorescence is an effective tool for sensing and assessing the impact of metals on the photosynthetic apparatus, used extensively to investigate effects of various toxins on Lemnaceae. Plant age and developmental stage strongly influence the effects of metals on the photosynthetic apparatus. However, there is little information on how this may affect toxicological assessment in plants. Zinc accumulation, morphological distribution and cellular storage, in either bound or soluble form, are also important for zinc sensitivity/tolerance and will be affected by increased zinc concentrations. Differential metal sensitivity of plant species has been linked with differences in accumulation and distribution. This study investigates how frond age and developmental stage, as well as zinc accumulation and storage, determine the impact of zinc on Lemnaceae. The maximum quantum efficiency of photosystem II, Fv/Fm, the effective quantum efficiency, Y(II), and photochemical quenching, qP, were measured in mature and young fronds as well as along a developmental gradient within fronds over seven days. Zinc accumulation and storage patterns (assessed as (i) zinc stored in fronds and roots and (ii) zinc stored in the soluble and bound forms) in the plants were also measured after seven days. L. punctata and L. minor accumulated more zinc in their tissues than L. gibba. Partitioning of zinc in L. minor was notably different from the other two species, zinc was stored mainly in roots and the bound-fraction. Fv/Fm and Y(II) in young fronds were more severely impacted by zinc than in mature fronds in all species. However, younger proximal sections of L. punctata fronds were more impacted than older distal frond sections compared to more homogenous impact in single Lemna fronds. Single colony, time-point or leaf-zone analyses may not show the full biological picture of the impact of a toxicant, especially not in L. punctata. Therefore, selection of Lemnaceae species and developmental stages of fronds should be given careful consideration when using chlorophyll-a fluorometry for assessing chemical toxicity in Lemnaceae.

TU 192

Alleviative effects of magnesium on copper rhizotoxicity to grapevine: macroscopic and microscopic point of view

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Intensive and long-term application of copper (Cu)-containing fungicides (e.g. Bordeaux mixture) in vine-growing areas has led to the accumulation of Cu on the surface of some vineyard soils around the world, thus posing the problems of Cu toxicity to the organisms therein. Recently, a number of studies have found that some cations, such as magnesium (Mg) and calcium, may alleviate the rhizotoxicity of Cu to plants. In this study, a hydroponic experiment was conducted to determine the influence of Mg concentration on the growth inhibition effects of Cu to grapevine roots. In addition, optical microscopy was used to examine the histological changes in root tissue at the cell level under Cu stress. Results indicated that the median inhibition concentration of Cu for root elongation increased significantly with increasing solution Mg concentration. On the other hand, the Cu concentration in roots was negatively correlated with the solution Mg concentration. From the light micrographs of root tip cells, the Cu concentration that caused histological changes in rhizodermal cells (i.e., increased vacuolization and plasmolysis) ranged from 10 µM, in the 0.2 mM Mg treatment, to 25µM, in the 4 mM Mg treatment. In the 8 mM Mg treatment, no histological change was observed even at an exposure concentration of 25 µM Cu. In conclusion, this study demonstrates that Mg can alleviate Cu rhizotoxicity to grapevines effectively.

TU 193

Metal effects in Fraxinus angustifolia and its endophytic communities

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Soil contamination with metals is a serious problem requiring urgent measures to counteract the impacts. The conventional soil remediation techniques are mostly inefficient and expensive. Phytoremediation comes as a promising alternative with a great potential for recovering ecosystem services. Besides looking for the best suitable plant, remediation programs should also focus on the microbial communities associated with the roots of the plants, namely endophytic and rhizosphere bacteria. In this work, we studied the potential of the species Fraxinus angustifolia for the phytoremediation of metal contaminated soils from the Cunha Baixa uranium mine (Mangualde, Portugal). The strategy consisted of the exposure of the plants to the contaminated soil, a reference soil and a control soil, for a period of about 3 months. To access the ability of F. angustifolia to tolerate the contamination and its physiological performance, a set of parameters (above-ground growth, leaf area, relative water content, maximum efficiency and quantum yield of photosystem II and leaf chlorophyll a and b, carotenoid, proline, and malondialdehyde contents) was measured during the test. Furthermore, the genetic profiles of the endophytic communities from the roots of the plants were also analyzed. This analysis was made via PCR-DGGE technique, targeted for a conserved region of 16S rDNA, pre- and post-exposure. Only above-ground growth was a suitable indicator of the negative effects of the contamination on the plants throughout the experiment. The remaining parameters were able to indicate the physiologic status, including recovery to a normal state. The comparison of the bacterial profiles pre- and post-treatment showed notable changes, mainly for plants exposed to the contaminated soil. The bacterial profiles from the control and reference soils showed more similarity with each other and with the pre-exposure profiles than with those from the contaminated soil. This study showed that F. angustifolia plants have the ability to resist and adapt to the adverse conditions of contamination, revealing a potential which can be exploited for phytoremediation. It also revealed that changes exerted on the bacterial root communities exposed to contamination, resulted in profiles considerably different from those of the remaining communities. The performance of the plants might be related to these microbial alterations.

TU 194

Dose-dependent effects induced by uranium (U) at pH 4.5 in Arabidopsis thaliana

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To evaluate the environmental impact of U-contamination, it is important to unravel the mechanisms by which plants respond to U-stress. It was already shown that U-exposure at pH 5.5 can disrupt the cellular redox balance and induce oxidative stress related responses in *Arabidopsis thaliana* plants (Vanhoudt et al., 2008). However, U-speciation and as such its toxicity strongly depend on environmental parameters such as pH. In a previous experiment, it was shown that U-toxicity at pH 4.5 was higher than at pH 7.5. Therefore, we want to investigate dose-dependent effects at low pH.

Arabidopsis thaliana plants were exposed to U-concentrations ranging from 0 to 100 µM at pH 4.5 during 3 days. U-concentration, fresh weight, lipid peroxidation and photosynthetic efficiency were analysed. On protein level, the enzymes of the antioxidative defence system were analysed to evaluate the importance of the cellular redox balance in *Arabidopsis thaliana* plants exposed to U.

Results indicate that the U-content in roots and shoots increased with increasing U-concentration added to the nutrient solution. However, there was a low root-to-shoot transfer. Fresh weight of roots and leaves decreased after exposure to 50, 75 and 100 µM U. In contrast, plants exposed to 6.25 and 12.5 µM U had an increased fresh weight as compared to the control plants, which alludes to a hormesis effect as was observed before (Vanhoudt et al., 2008, Straczek et al., 2009).

One of the most important physiological processes in plants is photosynthesis. Apparently, the photosynthetic efficiency of plants exposed to 25-100 µM U was increased. This indicates that the photosynthetic system is not damaged but in contrast plants try to optimize their photosynthesis under U-stress.

In addition to the reduced biomass production, plants exposed to 25-100 µM U showed an increased lipid peroxidation. This increase indicates an affected membrane integrity and functionality. Assessing antioxidative enzyme capacity of the plants indicated an increased defence against ROS in the U-exposed plants as evidenced by increased activities of ROS scavenging enzymes. The increased activity of guaiacol peroxidase could indicate an increased cell wall lignification as a defence reaction that limits the entry of toxic metals. In conclusion, this study indicates that elevated U-concentrations at low pH can cause important morphological, physiological and biochemical effects in *Arabidopsis thaliana* seedlings.

TU 195

The effect of pharmaceutical active compounds on oxidative stress response enzymes of the marcophyte Ceratophyllum demersum

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Pharmaceutically active compounds (PhACs) in the environment have recently been acknowledged to potentially constitute a health risk terrestrial and aquatic ecosystems and thus ultimately for humans. The occurrence and fate of selected human PhACs belonging to different therapeutic classes (non-steroidal anti-inflammatory drugs, anti-epileptics, contraception's and ß-blockers) have been detected in many countries in sewage treatment plant effluents, surface waters, seawaters, groundwater and some drinking waters. The biological effect of even low concentrations of PhACs on non-target organisms, such as aquatic macrophytes has so far received little attention.In nature aquatic organisms are constantly exposed to xenobiotic compounds that cause the overproduction of oxidants or reactive oxygen species (ROS) resulting in oxidative stress. Therefore, we have investigated the detoxification enzyme glutathione S-transferases (GST) in the plant extract to metabolize PhACs in vitro and the results shown the inhibition of GST activity under the influence of the used PhACs. The data of the peroxidase (POD) and catalase (CAT) suggests that PhACs do not directly cause oxidative stress by an increase in ROS production. Due to increased ROS accumulation PhACs may be selectively toxic to some organisms leading to ecosystem alterations.

TU 196
The toxicity of iron nanomaterials to aquatic and terrestrial plants
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Zero-valent iron (Fe0) as well as ferrate (FeV, FeIV) nanoparticles are well known especially for the ability to degrade a wide range of contaminants in both soils and ground waters. Although these materials are widely used in remediation processes across the world, almost no data are published about their ecotoxicological properties. This study aim was to provide new information about their effects on both aquatic (Lemna minor) and terrestrial (Sinapis alba) macrophytes. Our experiments show large differences in toxicity between tested species as well as oxidation state of nanomaterials. The higher an oxidation state the more pronounced toxicity and the higher EC50 values have been found, following the order as FeV > FeIV > Fe0. We used the frond numbers, root length and dry weight after 7 days of incubation as endpoints of the inhibition tests.

TU 197
Selection of suitable aquatic plants for phytofiltration of arsenic-contaminated water
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The work presented here is part of a larger on going study about the metals and metalloids accumulation in aquatic plants of uraniferous geochemical province of Central Portugal. It is oriented for the use of aquatic plants as indicators of metal contaminated waters and their potential use in phytoremediation.
In submerged and free-floating plants the concentrations of As were much higher than in emergent plants, with the exception of *Oenanthe crocata*. The highest concentrations of As were found in the submerged species *Callitriche brutia* (436.92 mg/kg DW), *Callitriche stagnalis* (354.03 mg/kg DW), *Callitriche hamulata* (160.37 mg/kg DW), *Ranunculus trichophyllus* (268.53 mg/kg DW), *Ranunculus peltatus* (103.98 mg/kg DW), in the free-floating *Lemna minor* (279.42 mg/kg DW), and in emergent plant *Oenanthe crocata* (157.94 mg/kg DW). The measured concentrations in most of the emergent plants, such as *Apium nodiflorum*, *Typha latifolia*, and *Juncus effusus* were significantly lower when compared with the previously species, even in the rhizomes/roots. Other metals, such as Cu and Zn, are also accumulated by plants from the Callitrichaceae family, namely, Cu by *Callitriche verna* and Cu and Zn by *Callitriche stagnalis* with maximum concentration values of 132 mg/kg and 1395 mg/kg in dry biomass, respectively.
The abundance of *Callitriche stagnalis* and the ability to accumulate arsenic and several heavy metals at the same time made this plant our first choice for rhizofiltration methodologies development.

TU 198
Genotipic variation in metal-tolerant Silene vulgaris clones
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Silene vulgaris is a facultative metallophyte with multiple tolerance and co-tolerance to heavy metals, capable of colonizing contaminated sites due to its fast and vigorous growth by producing seeds and rhizomes. In order to evaluate its potential for the purposes of phytoremediation, genetic approaches are required to uncover the allelic variation at both Mendelian and quantitative genes. Analitical tools based on the identification of useful genetic diversity are needed for plant genotype selection aimed at enhancing phytoremediation efficiency.
This study considers the genetic relationships among different *S. vulgaris* clones. Fourteen clones from ten populations collected from Madrid (Spain) were analysed using chloroplast DNA (cpDNA) markers. Ten primers pairs chloroplast SSR loci were tested and 3 out of the 10 primer pairs revealed the existence of intraspecific length polymorphisms. The intergenic spacer between the *trnH* and *psbA* genes was amplify with the primers *trnH* (GUG) and *psbA*. All PCRs produced a major robust band per primer pair in all samples studied. Two size variants were detected for ccmp1 (120 and 121pb), two for ccmp4 (113 and 114pb), five for ccmp2 (190, 191, 192,193 and 198pb) and six for *trnH/psbA* (328, 338, 349, 354, 355 and 357pb).
Regarding haplotipic diversity, ten unique cpDNA haplotypes were identified and seven of them were private, being found in only one population. Despite de limited number of clones examined, a considerable intraspecific polymorphism was detected and can be explained by *S. vulgaris* out-crossing breeding system. Conservation of genetic resources in this allogamous species requires maintenance of the genetic diversity within each clone to avoid inbreeding depression and loss of rare alleles.

TU 199
Biosorption of Pb by Chlorella vulgaris (Chlorophyceae) at laboratory scale
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In the lower Salado River (Santa Fe Province, Argentina) were reported levels of Cu, Cr and Pb in water, higher than permitted standards, thus highlighting the urgency of implementing remedial measures. Among the conventional methods to capture metal ions from aqueous solutions are ineffective when the volume of wastewater is high and the concentration of metal ions to be removed is low (1-100 mgL-1). An alternative is biosorption technology, using materials of biological origin. In the present study we analyze the efficiency of Chlorella vulgaris (Chlorophyceae) to remove Pb at laboratory scale.
The microalgae were harvested in exponential growth phase, centrifuged and resuspended twice in ultrapure sterile distilled water. After measure of algae concentration, we used three real concentrations of Pb+2 (Pb (NO3)2: 1.951 [C1], 2.826 [C2] and 4.830 [C3] mgL-1 with their respective controls, all in triplicate (50 mL-1). The vessels were maintained in an incubation chamber under controlled temperature (23±1°C), continuous illumination (3000 lux) and daily shaking. At 10 and 30 min, 1, 12 and 24 h of exposure, they were centrifuged for 10 min at 3000 rpm, separating the supernatant from the pellet of algae. Both matrices were analyzed by atomic absorption spectrophotometry. For each time and concentration, we calculated the % of removal, the concentration factor (CF= [metal algae] / [metal water]). Pearson correlations were performed to relate the concentration of Pb accumulated by C. vulgaris and the remaining in the supernatant.
C. vulgaris removed 50% and 47% of Pb at 10 min of exposure; 100% and 87% at 1 h exposure in C1 and C2 respectively. The maximum percentage (72 %) was obtained at 12 and 24 h in C3. After 12 h, we observed desorption of Pb, with concentrations ranging from 2.62 mgL-1 to 2.58 between 12 h and 24 h in C2. In C3, 3.27 mgL-1 (12 h) and 3.01 mgL-1 (24 h). The CF in C1 was >1 at 1 h of exposition, in C2 at 12 h was 0.93, falling slightly at 24 h. In C3 the CF was <1 in all the treatments, with higher values at 12 h (0.66-0.68) and decreasing at 24 h (0.61-0.63). There were significant negative correlations between the final concentration of Pb in algae and in the supernatant: r= -0.849, p<0.0001; r= -0.880, p<0.0001; r = -0.880, p<0.0001 in C1, C2 and C3 respectively. C. vulgaris is efficient to reduce Pb concentrations from the order of ppm to ppb from aqueous solutions, so it is recommended for wastewater treatment at a larger scale.

TU 200
Bioaccumulation and distribution of selected metals in the macrophyte Ceratophyllum demersum, in a polluted South African river
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Selected metals were measured in the submerged macrophyte Ceratophyllum demersum, introduced to the Diep River, Cape Town, South Africa. The Diep River passes various possible sources of metal pollution before reaching the Atlantic Ocean. Previous studies on this river have shown high levels of metal contamination, however, studies on metal bioaccumulation in aquatic biota such as plants are limited. Plants were obtained from a reference site and placed in baskets at two sites along the river (one upstream and one downstream from urban pollution sources). Plants, sediment and water were collected every fortnight for twelve weeks. Samples were analyzed for aluminium, iron, zinc and copper using nitric acid digestion and analyzed by means of ICP-MS. The results generally indicated that plants collected upstream had significantly higher concentrations of all four metals, compared to those collected downstream, possibly due to intensive agricultural activities upstream and the filtering effect of aquatic macrophytes between the two sites. Also, C. demersum generally rapidly bioaccumulated higher concentrations of metals in its leaves, compared to stems, possibly as a result of having a greater surface area for uptake. Additionally, metal-laden leaves may be shed as a detoxification method. In conclusion, it is clear that the metals in the Diep River are bioavailable for uptake in aquatic biota. Also, C. demersum may possibly be used in biomonitoring programmes. However, further research is necessary to investigate the toxic effects of the metals and how it relates to metal exposure.

TU 201
Selenium pathway in water moss Fontinalis antipyretica
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Water mosses are good indicator of polluted watercourses. We investigated the uptake of Se in widely distributed truly water moss *Fontinalis antipyretica* in its natural environment. Nine locations in Notranjska region, Slovenia, with different land use in the catchment, were sampled. Samples of water and moss were taken in all four seasons

during years 2009, 2010 and 2011. The concentration of Se in water from all locations did not exceed 0.2 µg Se L-1. *Fontinalis antipyretica* took up Se in the range between 343 - 3039 ng Se g-1 (on dry matter basis; DM). The Se content varied, depending on the location and season. The highest content of Se was measured in the stream of Žerovnišcica that flows through agricultural area with pastoral farming, the value being 3039 ± 170 ng Se g-1 (DM). The amount of insoluble Se compounds after enzymatic hydrolysis using Protease (XIV) was around 75%. In soluble Se compounds only traces of Se (IV) and Se (VI) were found. No other Se compounds (SeMeSeCys, SeMet) were detected.

TU 202
The organic matter contribution of salt marsh vegetation to coastal wetland ecosystems: a case study in Jiangsu, China
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Stable isotope techniques and different calculation models were used to analysis the potential sources of sediments organic matter in the core area of primary wetland and to calculate the contribution of salt marsh vegetation. By using multiple resources linear mixing model and the Euclidean distance model, we can conclude that micro-algae and Spartina alterniflora are the main contributors, under the assumption that the $\delta^{13}C$ value of micro-algae is -23", the average contribution rate of micro-algae is 40%, which is the main contributor to the salt marsh wetland ecosystems in northern Jiangsu. The contribution rate of Spartina alterniflora to its marsh is 56% and to the intertidal is 57%. By comparing the two quantitative models, we can find that the Euclidean distance model would overestimate the contribution of incidental resources and average the resource of high contribution rate.

TU 203
Genotoxicity study on Vicia faba L. grown on natural and spiked contaminated soils
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Contaminant bioavailability and toxicity varies among soil types and may depend on soil properties and on contaminants characteristics. Metals contamination could have different potential risks depending not only on the total content but on their bioavailability. Operationally, metal bioavailability is often evaluated by chemical extractions characterizing different chemical forms. The use of plant bioassay may be an amenable tool to screen the phytotoxicity of contaminated soils by metals.
In this work different soils contaminated by Boron (B) were studied in relation to plant bioavailability and toxicity. B is an essential micronutrient for plants and generally it is absorbed from soil in the form of boric acid. Previous studies demonstrated that both excess or deficiency of B can affect normal plant development.
Natural B polluted soils and artificially B spiked soils, with B total concentrations ranged between 20 and 100 mg/Kg, were tested on Vicia faba L., plant commonly used for detecting the genotoxic effects of environmental pollutants. Artificially contaminated soils showed the highest B bioavailability (about twofold) when compared with the natural polluted soil at similar B total content. Cytological analysis was carried out on root tip meristems of Vicia faba, after 3 days of seed germination in the different B polluted soils; mitotic index and micronucleus assay (MNC) were determined for genotoxicity evaluation. Moreover, developmental and physiological parameters were analyzed during plant growth in the B contaminated soils. The B content in distinct organs of the plants was determined and compared with B content in soil (distinguishing the total from the bioavailable) and with the genotoxicity effects.
The results showed the close relationship between B bioavailability, genotoxicity and B content in the plant. The natural polluted soil and spiked soil with similar B content in the bioavailable fractions showed the same pattern of phytotoxicity and genotoxicity in Vicia faba.
It is interesting to stress that bioavailability of B, and its content in the different plant organs, resulted higher in artificially spiked soils when compared with the same B concentrations in natural soils.
A close correlation between high B concentration in roots and major level of cytogenetic defects was found. These results confirmed plant genotoxicity of B polluted soils and showed clear evidences between soil properties, B bioavailability and phytotoxicity.

TU 204
Acute and chronic in vitro bioassays vs. autochthonous plants used as sentinel: comparing genetic biomarkers as tools in ecological risk assessment in Del Plata basin
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Del Plata basin is the fifth largest river basin in the world, its area covers four million km2 approximately and contains a great variety of natural environments and resources; these resources are shared by Bolivia, Paraguay, Brasil, Uruguay and Argentina.
The lack of wastewater treatments from 100 million inhabitants is the main source of chemical and biological pollution that impact on all tributaries and affects negatively to the biota.
Samples of surface river water from along 1200 km of river coast were analyzed according APHA, AWWA, WEF (1992) ;water quality indexes (WQI) were calculated Value were adjusted to Argentine guidelines and sampling sites were qualify, being Excellent (95-100) 11%, good (80-94) 21%, Fair (65-79) 26%,, Marginal (45-64) 26%and Poor (0-44) 16% of sampled places. Bioassays using water from Uruguay and Río de la Plata rivers were performed to evaluate toxicity. Allium cepa were exposed chronically and semi-statically while Latuca sativa assay was acute and static according IRAM standards. Samples of Echinodorus uruguayensis Arechav. (Alismataceae), Eichornia crasipes (Mart.) Solms (Pontederiaceae), Pistia statiotes L. (Araceae), Scirpus giganteus Kunth (Cyperaceae) were fixed in field to evaluate biomarkers. The biomarkers included in this study were chromosome count, chromosome characterization, occurrence of cytogenetic abnormalities and Mitotic Index calculated as (MI= # total of dividing cells x 100/nro total of observed cells) were studied. An average of 1800 + 500 cells were analyzed per individual. Uni- and Multivariate analysis were applied.
Pearson was a pristine site localized downstream of the Rio de la Plata and it was considered as reference point. While chronic bioassays showed higher MI in samples from Uruguay River, samples of water from the Rio de la Plata were characterized by abnormalities in mitosis. Classical end points might be masked by abundance of nutrients in water, in fact. differences were registered among, overpopulated and poor populated areas, industrial and agricultural developing areas. On one hand we found higher responses to multiple stressors in laboratory bioassays than in field specimens indicating an overestimation of water toxicity. Our results revealed that different sources of pollution depending on the characteristics of field could show similar results in some biomarkers in situ but integrating data with field results and WQI it could be possible to assess the ecological status.

RA20P - Risk assessment of chemical mixtures: where do we stand? what are the next steps?

TU 205
Can we possibly derive environmental quality benchmarks for chemical mixtures?
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In reality, many chemical pollutants are indeed coexisting in the aquatic environment. For example, many antifouling biocide residues are often detected as a cocktail in water and sediment samples collected from coastal environments. Based on literature review of documented studies on the combined ecotoxicity of antifouling biocides, we found that both additive and synergistic effects together account for 80% of all cases in which about 35% cases are synergistic. To allow more accurate risk assessment of concurrently occurring chemicals, there is a need to develop environmental quality benchmarks (EQBs) for their mixtures. In this presentation, I will introduce and discuss several possibilities of deriving EQBs for chemical mixtures. First, if all components in a chemical mixture are known to share a similar toxic mode of action, we can assume that the combined toxicity of the mixture would follow a simple concentration addition model, and the concept of toxic equivalency quotient (TEQ) could be applied to derive the EQBs based on lethal and/or effect concentrations expressed in terms of TEQ and/or TEQ concentration. This method has been applied to polychlorinated biphenyls, dioxins and dioxin-like compounds, and chlorinated polycyclic aromatic hydrocarbons. Second, if the mixtures contain chemicals with different toxic modes of action, it is possible to explore the use of the multidimensional species sensitivity distribution (m-SSD) approach. Here, I use binary mixtures of copper (Cu) and zinc pyrithione (ZnPT) as an example to illustrate the m-SSD method. We first conducted standard acute toxicity tests with an array of marine organisms for each chemical alone, and for their mixtures. The Cu-ZnPT mixtures showed a strong synergistic toxic effect to all test organisms. By utilizing all the toxicity data, we are able to construct a two-dimensional SSD in form of a response surface, from which we can derive any specific hazardous concentration (i.e., EQB) for the two compounds. This novel method can be potentially applicable to a more complex mixture by employing non-parametric response surface models. Third, I will highlight the field-based SSD approach, which is integrated with the quantile regression method, can be used to derive sediment quality guidelines for any target chemical with consideration of the presence of chemical mixtures and biological interaction. Finally I will discuss the pros and cons of each described method.

TU 206
Evaluation of available frameworks for mixture risk assessment in biocide and plant protection products in the EU
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Pesticides are regulated in the EU by the Biocide Product Directive (98/8/EC) (BPD) and the Plant Protection Product Regulation (Regulation (EC) No 1107/2009) (PPP Reg). A new Biocide Regulation (BP Reg) is proposed and will likely come into force during 2013. Environmental and human health risks are evaluated before a product may be authorised and sold on the European market. Simultaneous exposure to several substances based on the use of biocidal products and plant protection products are to be evaluated, according the PPP Reg and the BP Reg, respectively. This is more explicitly stated in the BP Reg compared to the BPD.
Guidance on how to perform mixture risk assessment on biocide or plant protection products is very limited. However, several frameworks for tiered risk assessment of the combined effect of substances within products have been suggested or are being developed by e.g. WHO-IPCS and Competent Authorities from several EU Member States, such as DE, DK, ES and SE.
In the present work several such frameworks have been evaluated for risk assessment of both environmental and human health. We have used model products which are based on actual biocide or plant protection products, and the outcomes of the evaluations are compared from a regulatory decision making perspective. The suggested frameworks are all based on tiered approaches that include various levels of precaution and conservatism in order to be sufficiently protective of human health and of the environment

even in data poor situations. The evaluation of the frameworks provides estimates of how protective and conservative they are in realistic regulatory assessments for both human health and environmental risk assessment.
The various frameworks and decision trees are easier to apply for risk assessment on higher biological organization, i.e. easier to apply to environmental risk assessment than to human health risk assessment. Suggestions on how to prioritise refinement efforts in the product dossiers should be included in forthcoming guidance.

TU 207
DPD+ - an appropriate method to assess the risk for mixtures under REACH?
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According to REACH chemical substances need to be registered at the European Chemical Agency in Helsinki. For hazardous substance produced in amounts above 10 t / y the manufacturer or importer of the substance has to conduct a risk assessment and document the assessment in a chemical safety report (CSR). Operational conditions (OC) and risk management measures (RMM) for the safe use of a substance are documented in exposure scenarios (ES). ES are attached to the CSR. In addition, manufactuer or importer have to create a safety data sheet (SDS) for substances to communicate conditions of safe use within the supply chain. ES are attached to the SDS. The formulators must assess the risk for the mixture they produce and create a SDS for the mixture on the basis of the information they get with the SDS and ES for the single substances. Although emissions to the environment results mainly from mixtures there is no guidance to assess the risk resulting from the use of mixtures. Industry developed the DPD+-method (based on the dangerouse preparation directive) to assess the risk from mixtures. Aim of the method is to consolidate the information in the SDS and ES of the single substances to derive conditions of safe use for the mixture. The method is based on the identification of a lead substance based on the classification of the substances in the mixture. Substances without classification are not considered in the derivation of OC and RMM for the mixture. The Federal Environment Agency, Germany (UBA) conducted a study to analyse the DPD+-method. The analysis tried to identify which potential risks of a substance could be overlooked by DPD+ and would hence not be adequately addressed in the selection of lead substances and the derivation of safe conditions of use. The above considerations question if DPD+ is a reasonable instrument to facilitate the consolidation of environmental information on mixtures. Proposals for possible improvements of DPD+ are mentioned above. This proposals need to be further elaborated and included in a method to assess the risk of mixtures. The interpretation of DPD+ (only substances with classified for environmental hazards need to be addressed in the risk assessment for the environment) is regarded as not in line with the interpretation of the legal text by Commission, ECHA and MS. Beyond that the possibility of an integration of scientific methods for the assessment of mixtures in the regulatory framework should be investigated.

TU 208
Application of the TTC Concept to focus cumulative exposure assessment in environmental media: a special case for pesticides?
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The concept of Threshold of toxicological concern (TTC) is based on establishing an exposure level for chemicals below which no significant risk is to be expected. This level is so low, that a risk assessment could be based only on structural or de-minimus considerations, even in the absence of effect data. Originally it was applied to toxicological evaluations of substances such as flavourings, food contact materials, and for the risk assessment of chemicals (EFSA, US FDA, WHO IPCS). Later it was adapted for ecotoxicology, and an "environmental threshold of no concern"(ETNCaq) of 0.1 µg/L was proposed for polar narcotics, non-polar narcotics and also reactive chemicals (de Wolf et al. 2005). However, because of their specific MoA pesticides were considered as different and some showed lower NOECs and thus were not included.
The TTC concept could be helpful to focus the assessment of chemicals that occur in environmental media. The recent SCHER opinion on Toxicity and Assessment of Chemical Mixtures recommended that no further risk assessment is needed for substances that do not exceed the TTC. Thus the TTC could serve as a filter to avoid making mixture toxicity assessment unnecessarily complex and laborious by including a multitude of substances which are not relevant for the assessment.
An analysis of the Bayer CropScience portfolio suggests that regulatory acceptable concentrations (RACs) for most pesticides are well above the ETNCaq of 0.1 µg/L suggested for chemicals other than pesticides. However, the original application of the ETNCaq was to derive safe thresholds in the absence of effect data, whereas for pesticidal active substances robust acute and chronic ecotoxicity data are always available. Thus the limited number of substances that have lower threshold of concern can easily be identified and should then be included separately in a mixture toxicity assessment, even if present at concentrations below the ETNCaq. The application of the ETNCaq as a generic TTC in the field of mixture toxicity assessment in environmental media would primarily serve to filter out irrelevant contributors, not to substitute measured effect data.
That TTC for surface water could also guide where to set the generic LOD in monitoring studies rather then to try to detect substances at levels "as low as possible". Further analysis is needed to assess whether a generic TTC of 0.1 µg/L is suitable and this should be tested on relevant real-world cases.

TU 209
Relevant potency threshold: reducing uncertainty by calibration of cumulative risk assessments
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Relative approaches, i.e., toxic equivalent (TEQ)-like approaches, for mixtures risk assessment have been established for some time. TEQ-like approaches assume that if certain premises are met, e.g., chemicals are true congeners, are metabolized and detoxified by the same biological processes, produce the same spectrum of biological effects by the same mode of action, and exhibit parallel dose response curves for the biological effect being modeled (Safe 1990), then those chemicals may be assumed to be dose additive (DA) for specific toxic effects. In 2008, the National Research Council published a report entitled " Phthalates and Cumulative Risk Assessment: Tasks Ahead, " containing recommendations for cumulative risk assessment practices at the U.S. EPA. The Panel rejected the underlying premises of TEQ-like approaches, asserting that cumulative risk assessment should be conducted by applying DA to chemicals that produce " common adverse outcomes " (CAOS) rather than to chemicals with similar modes or mechanisms of action. Using the data available, published mixtures data upon which NRC based its recommendations and a published human health risk assessment that used the DA-CAOS concept were evaluated to determine how firmly the concept is supported by the data and with what level of certainty the results may be extrapolated to lower doses and to humans. The underlying assumptions of the model and the risk assessment were probed for consistency with available human data, using data for two human pharmaceuticals as test cases. P redictions based on the DA-CAOS concept were compared with human clinical experience to determine whether those predictions are verifiable and consistent. Based upon this analysis, an alternative approach was developed - the Human-Relevant Potency-Threshold (HRPT) - that appears to fit the data better and avoids the contradictions inherent in the DA-CAOS concept. The proposed approach recommends application of independent action for phthalates and other chemicals with potential anti-androgenic properties at current human exposure levels. This example should be instructive for the inevitable discussion of the suitability of DA-CAOS for addressing environmental cumulative risk assessment for hormonally active agents, and environmental mixtures in general.

TU 210
Application of delayed fluorescence to estimate influence of chemical mixtures on alga
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Algae are important primarily producers in aquatic ecosystems. The OECD Test Guidelines 201 (TG201), alga growth inhibition test, is a conventional method that evaluates chemical toxicity on alga. The conventional method evaluates influence of chemical substance on algal growth from a 72 h exposure. In the study of chemical mixtures, a method that can estimate chemical toxicity in shorter time or provide information on the mode of action of chemical substance would be advantageous. Applications for a new method include improving the evaluation efficiency for toxicity screening of a large number samples for regulatory filings, toxicity identity evaluation of chemical mixtures, and assessment of effluent toxicity. We are investigating a new rapid estimation method of the growth inhibition using delayed fluorescence (DF) from algae. The DF is a special type of luminescence; it detects the growth of only those cells that have photosynthetic capability. Since the DF originates from re-excitation of chlorophyll by a reverse reaction of photosynthetic electron transfer, the DF inhibition reflects inhibition of photosynthetic activity that is necessary for growth. Therefore the DF is a potential endpoint for estimation of the influence of chemical substance of algal growth in a shorter time than the conventional 72 hours growth inhibition test (e.g. TG201). In addition, the intensity of DF shows a time decay curve. Chemical exposure changes the decay curve to patterns that provide insight into the mechanisms of action, and also has potential for application to the identification of contaminants in mixtures. We discuss characteristics of the evaluation of the influence of chemicals (herbicide, other chemical compounds and its mixtures) on alga by both the DF inhibition and analysis of the DF decay curve. We also compare the detection characteristics of DF and other rapid estimation methods, including chlorophyll fluorescence-based photosynthetic activity measurement (PAM). and Microtox®, a common rapid bioassay that uses luminescent bacteria to evaluate complementary sensitivity between photosynthetic and non-photosynthetic microbial bioassay

TU 211
Species sensitivity distribution for the prediction of herbicides mixtures toxicity on benthic diatoms
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Coastal zones of lakes could be contaminated by a cocktail of substances coming from urban discharges or diffuse watershed run off. This type of contamination especially concerns micro pollutants such as pesticides. Benthic diatoms, which are a major component of benthic biomass, are then exposed to several pesticides that could interact together and present a modified toxicity (synergy, antagonism). Considering risk assessment, Species Sensitivity Distribution (SSD) models are partly used to extrapolate protective concentrations for a community exposed to a single or a cocktail of contaminants. To predict the toxicity of a mixture, two concepts are used depending on the mode of action of the mixture components (Concentration Addition _CA, or Independent Action_ IA). The main objective of the study was to assess 1/ if SSD (using CA or IA models) were reliable in terms of toxicity prediction of herbicide mixture on benthic diatoms and 2/ if this SSD could be used as a management tool for aquatic ecosystems. As a first step, eleven species of benthic diatoms were exposed to 4 herbicides, separately (diuron, isoproturon, terbutryn, atrazin), in 96h monospecific growth inhibition tests.

EC_{50} values derived from dose-response curves allowed to build a SSD curve (SSD-EC_{50}) for each herbicide. The different individual SSD-EC_{50} curves were combined, using CA or IA hypothesis, in order to predict the toxicity of several mixtures. This first step allowed us to predict hazardous concentrations (HC).
Then each species was exposed to three different mixtures (96h monospecific growth tests). Two binary mixes (atrazin/terbutryn; diuron/isoproturon) and one quaternary mix (diuron/isoproturon/terbutryn/atrazin) were tested. EC_{50} obtained for each species-mixture combination allowed to build a SSD curve (SSD-EC_{50}) for each mixture. Measured HC were obtained from these curves.
Finally, we compared the toxicity thresholds predicted by CA and IA models to the measured toxicity thresholds. We also assessed the species ranking between the mono herbicide SSD curves and the mixture SSD curves.

TU 212
Effects of Atrazine and 2,4-D mixtures on lemna minor
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Thailand is country where agriculture is very important in terms of the economy. Pesticides are therefore widely used in Thailand and this usage in increasing. As a wide range of pesticides is used in Thailand, aquatic will be exposed to a mixture of compounds. In order to understand pesticide impacts, it is therefore important to understand the potential toxic interactions of pesticides in use in Thailand. The aim of the present study was therefore to test the interactive effects of two herbicides, atrazine and 2,4-Dichloroacetic acid which are some of the most widely used and imported pesticides in Thailand. The test was carried out on Lemna minor.
The effects of atrazine and 2,4-D on their own and in mixtures were assessed using OECD method 221. For the single compound studies, the effects of a range of concentrations of pesticide on the growth of L. minor were studied. For atrazine the test concentrations were 0.05 ,0.1 , 0.2 , 0.4 and 0.80 mgL-1 and 2,4-D were 5 ,10 , 20, 40 ,80 and 100 mgL-1 . L. minor one colony, 3 healthy fronds was exposed to the test solutions for a period of 7 d. The design of the mixture studies was used seven mixture ratios and seven chemical dilutions. Dose response curves were obtained and fitted in logistic regression by using sigma plot. Isobole analysis was used to interpret the mixture toxicity results.
Results from the single substance studies showed that atrazine was more toxic to L. minor than 2,4-D. Due to 2, 4-D being an auxin hormone, it is absorbed through the leaves and translocated to the meristems of the plant in dicots and it does not affect monocots which may explain the low toxicity to L. minor. The studies into the combination effects of atrazine and 2,4-D on L. minor cleary showed that the two compounds interact in an antagonistic manner. The antagonism has been found to occur more frequently in mixture where the herbicide mixture belongs to different chemical groups and monocot species.
In terms of this mixture study, the results showed that the interaction between atrazine and 2,4-D was antagonistic. These results are re-assuring and indicate in terms of impacts on aquatic macrophytes in Thailand, mixture effects are less than additive. If additivity is assumed for risk assessment purposes for macrophytes in Thailand, then this should be protective. Work is ongoing using a wider range of pesticides and test organisms.

TU 213
Predictive mixture toxicity assessment of pesticides in Swedish surface waters
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It has been demonstrated that combined effects of chemical mixtures give rise to stronger toxic effects than any of the compounds applied individually. The Scientific Committee on Health and Environmental Risks (SCHER) has recently put forth concentration addition as a conceptual basis for the determination of environmental quality standards for mixtures. In this study we have predicted the risks from pesticide mixtures within Swedish surface waters using the concentration addition concept. Data from the national pesticide monitoring program in Sweden has been combined with experimental data from the US EPA's database for single chemical toxicity and predicted toxicity from QSAR's to determine the risks from pesticide mixtures in Swedish surface waters close to agricultural areas. The Swedish monitoring program has been taking samples in six different locations since 2002 and currently analyses the samples for more than 80 compounds, out of which 78 has been detected. All and all 751 weekly samples have been analysed bringing the total number of analyses performed close to 60 000. The abundance of information enabled the study of not only the individual site risks but also additional questions. Among these the effect on risk from season, taxa most at risk, average error factor when ignoring mixture effects as well as the sensitivity of the risk assessment to different ecotoxicological data has been determined. The results show that the average risk quotient of the mixtures from the six different sites range between 2,3 and 18,9 thus calling for additional assessments. The study also show no effect on risk from season implying that potential for recovery from pesticide stress is very limited. Finally the high ratio between predicted risk of the mixture and the average risk of the individual substances clearly demonstrates the need for going beyond the standard compound-by-compound assessment.

TU 214
A proposal for considering mixture toxicity with EQS compliance checking
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Environmental quality standards (EQS) are used under the EU Water Framework Directive to determine the chemical status of a water body. At present, compliance with EQS is only checked on the single substance level. However, a large and growing body of evidence from mixture toxicity studies indicates that this procedure might underestimate the toxicity of the chemicals present in a water body, as the effect of mixtures has been shown to be higher than the highest single substance effect in the majority of cases. This propensity to underestimate potential effects of mixtures is underlined by a recent review published by Kortenkamp and co-workers. Two approaches have been proposed to date to account for mixture toxicity in assessing the chemical status: (i) application of a default assessment factor of 100 as used for EQS derivation by RIVM (NL) and (ii) application of mixture toxicity models based on species sensitivity distributions (SSD). The latter method predicts the 'multi-substance potentially affected fraction' (msPAF) of species proposed by Posthuma and co-workers. In this paper we propose a third approach for cases where the EQS was not derived from SSDs for all mixture components, which allows more accurate assessment of environmental mixtures even where there is incomplete SSD information. The approach is based on the identification of the most susceptible taxonomic group and can be combined with the mixture cumulative ratio [MCR] approach recently proposed by Price and Han. It will also take into account the fact that specifically acting substances will only be specifically toxic to some taxonomic groups while exerting baseline toxicity to other taxonomic groups. This novel approach will be illustrated using case studies based on real monitoring data and published EQS values. The everyday applicability of the approach will be discussed along with the decrease in uncertainty that it achieves when assessing the chemical status of water bodies containing more than one substance of concern.

TU 215
Increasing the number of data points does not necessarily reduce the probability of erronously conlusions about interactive effects in mixture toxicity experiments
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The importance of mixture ecotoxicity studies is increasing exponentially. Developing requirements in regulatory risk assessment are further fuelling the need to gain more knowledge about mixtures, mixture experiments and the subsequent data analysis. In particular, little attention has been given to the choice of an appropriate experimental design for a given reference model (e.g. independent action and concentration addition). Indeed, it has been shown that some experimental designs might not be able to detect (false negative) or erroneously conclude (false positive) deviations from a reference model. In this study, we simulated experimental data from a full factorial and equitox design and subsequently tested if these data deviated significantly from the concentration addition reference model. Experimental data were simulated for synergistic, antagonistic and additive mixtures. We then determined false positive and false negative rates for each experimental design. The number of data points, the lowest concentration of each chemical and the step size between two subsequent concentrations were varied. Results demonstrated a marginal increase in false positive rates with increasing number of design points. This contrasts with the general belief that adding design points will reduce the false positive rate. Varying the two other parameters resulted in a significant decrease in false positive rates. An optimal combination of these two parameters leads to the lowest false positive rates for a given design. In conclusion, we have shown that an optimal experimental design for mixture toxicity focuses on optimizing the lowest tested concentration and the concentration step size rather than increasing the number of design points. Furthermore, datasets based on suboptimal or imperfect designs lead to erroneous conclusions and care should be taken in analyzing and evaluating such datasets.

TU 216
Proposed PAH relative potency factors will greatly increase risks at all sites with PAH mixtures
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The United States Protection Agency (USEPA) is proposing to modify the approach that it has been using since 1993 to evaluate polycyclic aromatic hydrocarbon (PAH) mixtures. While USEPA previously considered seven PAHs to have carcinogenic potential, the proposed approach increases the number to 26. In addition, many PAHs are being assigned a Relative Potency Factor (RPF) that is greater than 1 relative to benzo[a]pyrene. Several proposed RPFs are greater than 10, including benzo[c]fluorene (20), dibenzo[a,l]pyrene (30), and benz[j]aceanthrylene (60). If this approach is adopted, it will be necessary to quantify all 26 compounds in media at contaminated sites, despite the lack of standard analytical methods. In addition, it may be necessary to re-open and reassess sites that have already received regulatory closure. However, there are many scientific criticisms of the proposal. Most importantly, no Weight of Evidence Evaluation was performed. Second, USEPA assumed that all PAHs act by a similar mode of action with no supporting evidence. USEPA also failed to validate the derived RPFs using cancer response data from real world complex mixtures, such as coal tar. This paper summarizes several validation exercises that demonstrate that USEPA's RPFs overestimate the true carcinogenic risk observed when the interactions between components are inherently taken into account in mixture studies. Lastly, there were many technical problems with proposed RPFs: many RPFs were based on a single study or "low confidence"studies; some RPFs were based on in vitro assays; some RPFs were derived from studies with no confirmation of the chemical identity of the test substance; some RPFs were based on studies with unusual modes of administration, such as lung implantation; and many studies exceeded the Maximum Tolerated Dose. This paper presents the proposed approach to evaluating PAH mixtures and discusses key scientific criticisms.

TU 217
Derivation of environmental risk limits for polycyclic aromatic hydrocarbons (PAHs) based on internal residues
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Polycyclic aromatic hydrocarbons (PAHs) are substances that have both natural and anthropogenic origins. They can be formed as a result of combustion, and are constituents of many petroleum products as well. Environmental risk limits (ERLs) were derived for 16 PAHs that were also considered in the European Risk Assessment Report (RAR) on coal tar pitch, high temperature, but completed with a substantial amount of additional data.
It can be assumed that toxicity of all PAHs is similar and possibly caused by narcosis. The toxicity of different PAHs differs only as a consequence of different environmental distribution and accumulation potential. The sum of the internal concentrations of different compounds gives rise to the same effect as that of a similar concentration of an individual compound, which is referred to as concentration additivity. This concept has been succesfully applied before for total petroleum hydrocarbons (TPH) and will be investigated here for PAHs.
All ecotoxicity data were collected and carefully evaluated for their usefulness and reliability. To calculate the total internal residues, pore water concentrations were calculated first for soil and sediment, by considering partitioning between organic carbon and water. From water concentrations, the internal residues were calculated using a partition coefficient between the membrane and water.
From all chronic toxicity data for individual PAHs expressed as internal residues, a set of no observed effect residues (NOERs) for 54 species was obtained that showed no significant differences between the compartments (e.g. between aquatic, benthic and terrestrial species) and between the individual PAHs, which confirms the assumption that indeed accumulation from (pore) water is the determining factor for toxicity. On basis of these data, a species sensitivity distribution (SSD) was constructed, which appeared to be very similar to the SSD for TPH, suggesting a similar mode of toxic action. To derive a value for a generic ERL, an assessment factor of 5 has been applied to the HC5 to account for the potential of certain PAHs to exert a high acute toxicity through phototoxicity.
For the PAHs it is confirmed that the equilibrium partitioning method is a useful method in setting quality standards. Because toxicity is driven by equilibrium partitioning, monitoring of these PAHs could be focused on measuring free water concentrations, e.g. in pore water, with solid phase extraction techniques.

TU 218
Prediction of the mixture toxicity of PAHs and their derivatives in the Ah receptor based H4IIE-luc assay
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Polycyclic aromatic hydrocarbons (PAHs) form a large group of widespread pollutants of high environmental concern. Many of the PAHs are toxic and exposure can result in mutagenesis and carcinogenesis in humans and animals. PAHs are transformed to oxygenated PAHs by chemical reactions in the air and metabolic reactions in organisms. Oxygenated PAHs (oxy-PAHs) are mutagenic and in addition more mobile than their parent PAHs. Today, the generic guideline of PAH contaminated areas are based on the 16 U.S. Environmental Protection Agency (EPA) priority PAHs, even though often hundreds of PAHs and PAH derivatives exist in the soils. Aryl hydrocarbon receptor (AhR) mediated toxicity of PAH contaminated samples has been shown in several studies, but knowledge of the mixture interactions is insufficient. The aim of this study is to investigate the predictability of the AhR mediated activity of mixtures containing both oxy-PAHs and PAHs, using the concentration addition model (CA). It will be done by testing 26 individual PAHs and 12 oxy-PAHs and different mixture combinations of these compounds in the Ah receptor based H4IIE-luc assay. The observed mixture toxicity will then be compared with the mixture toxicity predicted by the CA model, assuming for additivity. The results presented here will aid future research using mass balance analysis of environmental samples and bring additional knowledge regarding mixture effects of PAHs and PAH derivatives via the Ah receptor.

TU 219
Hazard evaluation of shale oil to the environment
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Estonia is the country with the largest commercially used oil shale basin. Oil shale is used for the firing of local power stations but also for the production of shale oil. The latter is used in boiler houses, industrial furnaces but also for heating of houses, etc. The current annual production volume of shale oil is about 0.5 million tons and that will be increased up to one million tons per year in the near future. Increased production, transportation and use of shale oil in Estonia entail risks of environmental contamination. Different shale oil fractions contain various hydrocarbons, phenols, sulfur- and nitrogen-containing compounds, etc. The knowledge of shale oil toxic properties and behaviour in the soil and water ecosystems is limited. This current study aims to contribute to filling of these data gaps. In order to study the mobility and fate of the shale oil, the spiked soil samples were incubated in laboratory (i.e., controlled test conditions) and outside (natural climatic conditions) for several months. The dynamics of chemical composition of spiked soils was followed using different analytical methods. Potential hazard of shale oil to terrestrial (soil microbial community, higher plants) and aquatic organisms (bacteria Vibrio fischeri and crustaceans Daphnia magna) was investigated using various test formats.
The assessment of risks related with contamination of soils and surface waters by different fractions of shale oil based on the obtained results will be presented. This research is supported by Central Baltic INTERREG IV A Finnish-Estonian project: Risk Management and Remediation of Chemical Accidents.

TU 220
Application of predictive mixture models to differentiate P-gp and MRP type efflux transporter activities in zebra mussel (Dreissena polymorpha) and its environmental implications
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Aquatic organisms have developed strategies to cope with a variety of natural and anthropogenic toxicants. A central defense mechanism is the *Multi Xenobiotic Resistance* (MXR) system. MXR is mediated by transmembrane transport proteins belonging to the ABCB, ABCC and ABCG sub-families of the *ATP binding cassette* (ABC) superfamily of proteins. ABC transporters translocate their substrates in an ATP-driven process out of the cell. MXR transporters are particularly abundant in tissues with a barrier function, such as gills in mussels.
This work aimed at identifying P-glycoprotein (P-gp; ABCB subfamily) and multidrug resistance related protein (MRP; ABCC subfamily) efflux activity in gill tissue of zebra mussel (*Dreissena polymorpha*) and determining inhibitory effects of chemicals on P-gp and MRP efflux activities. Calcein-AM, a substrate of both pumps, was used as proxy dye for P-gp and MRP activity along with the specific inhibitors Reversin 205 for P-gp and MK571 for MRPs. Thus, a calcein-AM uptake assay was applied to study single and combined effects of the inhibitors on the efflux transporters. Enhanced accumulation of calcein in the gill tissue as reflected by increased calcein fluorescence indicated decreased efflux activity. Next, joint effects were modeled using the independent action and concentration addition concepts. Reversin 205 and MK 571 showed a distinctive calcein accumulation kinetics and their joint effects were greater than those predicted by the independent action concept. This indicates that the studied inhibitors targeted different P-gp and MRP efflux pumps but act in a cooperative manner in *Dreissena* gills. Furthermore the ability of twelve pollutants to inhibit P-gp and MRP pumps was tested. In addition to calcein-AM, also assays with rhodamine B, supposedly a substrate of P-gp but not MRP, were performed. Most tested pollutants had an equal or greater inhibitory effect on rhodamine B efflux than on calcein-AM, which indicates that they are specific P-gp inhibitors. However, e.g. perfluorooctane sulfonate (PFOS) had a greater inhibitory potential when calcein-AM was used, pointing to specific inhibition of MRP pumps by this chemical. This is the first study attempting to distinguish between inhibitory effects of environmental chemicals on either P-gp or MRP type efflux activities in a freshwater mussel.

TU 221
Characterization of the multixenobiotic resistance (MXR) mechanism in Daphnia magna and studies on its role in tolerance to single and mixtures of toxicants
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The study of the cellular mechanisms of tolerance of organisms to pollution is a key issue in aquatic environmental risk assessment. Recent evidences indicate that multixenobiotic resistance (MXR) mechanisms represent a general biological defense of many marine and freshwater organisms against environmental toxicants. In this work, the detection and functional characterization of toxicologically relevant efflux transporters were studied for the first time in Daphnia magna. P-gp and MRP transporter gene expression was studied by RT-PCR, and its associated efflux activity was monitored using the fluorescent dye rhodamine 123 combined with specific MXR transporter inhibitors (chemosensitizers).
Toxicity bioassays with model substrates of P-gp (mitoxantrone) and MRP (chlorambucil, mercury) applied singly and in combination with different chemosensitizers were performed to elucidate the tolerance role of P-gp and MRP efflux transporters.
Results provide evidence for the existence of the genes and the associated efflux activities of ABC transporters in juvenile stages of D. magna. Specific inhibitors of P-gp and/or MRP transport activities including cyclosporine A and verapamil but not MK571 and reversin 205 resulted in a dose dependent inhibition of rhodamine 123 transport in D. magna juveniles. Binary mixtures, of the tested transporter inhibitors, with the pharmaceutical drugs and P-gp substrates chlorambucil and mitoxantrone and the MRP substrate, mercury, increased the toxicity of the studied pollutants more than additively as predicted by the independent action concept. These results indicate that MXR transporter activity is present in D. magna and that this may play an important role in its tolerance to environmental contaminants.

TU 222
Validation of the simulated earthworm gut (SEG) for widespread use in soil invertebrate toxicity testing
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The Simulated Earthworm Gut (SEG) is an in-vitro test that can be used to estimate the bioaccessibility of contaminants found in soil. The main objective of this research is to validate the SEG as an indicator of soil invertebrate toxicity for soil contaminated with petroleum hydrocarbons (PHCs). Current practices for determining site-specific toxicity for soil organisms can be expensive and time consuming. By validating an in-vitro toxicity test such as the SEG, site specific remedial objectives can be determined faster and at a lower cost. To validate the SEG, we compare the toxicity of six PHC contaminated soils for three invertebrate species (mite, collembolan and earthworm) against the bioaccessible estimate obtained from the SEG. Furthermore, due to the lipophilic nature of hydrocarbons we compare SEG bioaccessibility with and without the addition of a lipophilic sink to see which model better characterizes in-vivo exposure.

TU 223
Prediction of mixture toxicity for metals in soil: a reality-check
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Today, EU Chemicals legislations are based predominantly on assessments carried out on individual substances. However, mixture toxicity is an upcoming issue in the regulatory area because of growing concern about the potential adverse effects of interactions between substances when present simultaneously. Only a few models are available so far to predict mixture toxicity (dose/concentration addition and independent action), and dose/concentration addition seems to be commonly selected as a default for regulatory purposes because of its higher protection level and the limited information about the mode of action of most substances. The applicability of these models in a regulatory framework for assessing the ecological effects of naturally occurring elements, such as metals, in soil may not be straightforward, however. For several metals, the predicted no-effect concentrations are close to their natural background concentration in soil, and adding the potential effect of several metals may lead to over-protective results and e.g. identification of risks in uncontaminated natural soils. Consequently, a reality-check is carried out on the use of common models for assessing the mixture toxicity of metals in soils and the selection of the number of metals to be included, based on exposure data for several metals in arable land and grassland soils across Europe (data from the GEMAS project). The distribution of the predicted total risk ratio across arable land or grassland in Europe is evaluated with respect to the mixture model applied, the number of metals included, the incorporation of bioavailability corrections, etc.

TU 224
Toxicological evaluation of chemical mixtures posing hazard to human and environment
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Exposure of human and other organisms to chemical mixtures found in an environment can occur in different forms, with regard to reason of the contact and composition of the chemical mixture. Beyond all doubts is the fact that the chemical risk assessment, due to higher and higher contamination of environment with multi-component chemical mixtures, plays crucial role in toxicology and ecotoxicology.
This work ilustrates the most important issues concerning toxicology of chemical mixtures, with reference to an experimental design and special attention paid to the types of study methods, mainly acute and chronic studies, as well as legal aspects relating to European regulations connected with REACH and CLP.

TU 225
Assessing human health risk from farmed milkfish consumption: considering toxic interaction among arsenic, copper, and zinc mixture
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Various studies indicated that a strong association between cultured fish and waterborne metals of arsenic (As), copper (Cu), and Zinc (Zn) in coastal areas of Taiwan regions. Generally, the impact of mixture toxicity of metals on the aquatic ecosystems is higher than that of single metal. The purpose of this study was to assess the human health risk for toxic interactions among As, Cu, and Zn mixture from farmed milkfish (Chanos chanos) consumption in blackfoot disease (BFD)-endemic area in Taiwan. The interaction risk assessment model proposed by the U.S. Environmental Protection Agency (EPA) and Agency for Toxic Substances and Diseases Registry (ATSDR) was used to estimate the interaction-based hazard index (HIINT) of non-carcinogenic mixture toxicity. The mixture toxic interactions of As'Cu and As'Zn were both antagonistic, whereas Cu'Zn was no significant influence. The 90th percentiles of HIINT for As'Cu, As'Zn, and As'Cu'Zn ranged from 0.74 to 1.78, 0.37 to 1.37, and 0.73 to 2.53, respectively. Our study indicates that As, Cu, and Zn mixture exposure may pose potential non-carcinogenic risk to human consuming farmed milkfish. The present interaction risk assessment model provides an illustration for assessing the potential threat to human health posed by the mixture toxicity of metals from seafood consumption.

TU 226
Whether BDE209 interacts with Cd hepatotoxicity?
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During the last ten years, mixture toxicology has undergone a remarkable and productive development. In fact, combined toxicity, which more accurately represents real environment conditions, may assist in the determination of toxicologically relevant effects. The aim of this study was to examine whether decabromodiphenyl ether (BDE 209) influences cadmium (Cd) hepatotoxicity.Wistar rats (200-240 g) were exposed orally to BDE 209 (1000 mg/kg/bw), Cd (2.5, 7.5 or 15 mg/kg/bw) or their three combinations, by gavage, for 28 days. Control groups were exposed to saline or DMSO as vehiculum. The following end points were examined: liver weight, morphology, histology as well as liver enzymes activities (aspartate aminotransferase-AST, alanine aminotransferase-ALT and γ-glutamyltransferase-γ-GT). Study was approved by Ethical Committee of Military Medical Academy (No. 9687/11).Liver weight increased in all groups comparing to controls. Interestingly, the highest increase was seen in Cd groups (20-42%), than in BDE209 group (28%), while in mixture groups it ranges between 96 and 115%. Morphological changes in liver were not observed while histological analysis indicated degenerative changes in hepatocytes, hyperemia, vascular bleedings and disruption of vascular membranes. Average histological scores on scale from 0 to 5 were: 0.13, 1.26, 2.26, 3, 3.4, 2, 2.03 and 2.26, for control, BDE2091000, BDE2091000+Cd2.5, BDE2091000+Cd7.5, BDE2091000+Cd15, Cd2.5, Cd7.5, and Cd15, respectively. AST values in general did not change, AST decreased, but more in single compound groups than in mixtures. Furthermore, ALP was significantly lower in Cd groups, while in mixture and BDE209 groups decrease in ALP activity was observed, but not so intensive like in all three Cd groups. Activity of γ-GT was higher in the mixtures than in groups where Cd or BDE209 were administered alone. Watching scores of histological changes and γ-GT activity, we may assume that BDE209 potentiate Cd effects on liver.(Partly supported by Ministry of Science project III46009)

TU 227
Waste recovery - a special treatment under REACH
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According to REACH, a manufacturing process is considered any production or extraction of substances in the natural state. This includes chemical modification as well as mechanical processes. Although the entry products at recovery companies are mainly waste (covered by the Waste Regulation), the status of the end products might "cease to be waste", and thus fall under the REACH regulation, and thereby having a major impact on the legal obligations of the recovery operator. However, under certain conditions recovery operators can benefit from registration exemption by applying Article 2.7(d) of the REACH regulation.
As the end products at recovery plants are often complex mixtures containing substances whose presence is not always intended, the process of substance identification is challenging. Each compound in the mixture has to be identified as either substance (mono-constituent, multi-constituent or UVCB), or impurity.
Once it is established that Art.2.7(d) can be applied, the recovery operator needs to fulfill certain obligations, such as the derivation of the classification of substances and mixtures, the notification of substances, the preparation of Art.2.7(d) dossiers (substantiating the application of the exemption article) and the development of SDSs. Obviously, little information is available on the mixture itself due to the often changing composition, nor on the impurities due to limited access to the raw data as recovery operators are not participating in SIEFs or consortia. This lack of data influences the derivation of C&L of the mixture since this will often be derived based on the impurities' classification alone (e.g. summation method). Furthermore, with the exemption from registration, the recovery operator is not required to perform an exposure and risk assessment.
One should stimulate the re-introduction of former waste products on the market, because this contributes to the recycling of natural resources and the products may have economic benefits in the time of raising commodity prices. Nevertheless, sufficient efforts should be made to assure safe use of these, potentially heavily classified, mixtures. In addition, the hazard and risk assessment should be performed in such a way that overestimation due to the application of conservative assumptions and worst-case scenarios is avoided. Although these concerns mainly address issues related to metal-based waste products, some may also apply to other types of recycling industries.

TU 228
Toxicity evaluation of disperse red 1 textile dye using freshwater organisms from different trophic levels - a PNEC proposal
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Azo dyes have been found in surface water especially because of textile production; however data about ecotoxicity of dyes are scarce in the literature. Commercial azo dyes are mixtures of a main dye, surfactants and other synthesis impurities. The commercial dye Disperse Red 1 containing 60% of the main dye N-Ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline, CAS number 2872-52-8, was obtained by PCIL industries Ltda, Brazil. This product is used for dyeing synthetic fibers. Previous data showed the main dye was mainly responsible for the commercial dye toxicity at least for Daphnia. This same dye was found in an effluent and a receiving water sample collected in Americana region, São Paulo Brazil. The aim of this study was to compare the results of ecotoxicity of the commercial dye Disperse Red 1 obtained for organisms from different trophic levels and propose a PNEC (predicted no effect concentration) for aquatic life. For chronic tests, the NOEC (72h) obtained for Pseudokirchneriella subcapitata was 0.1 ppm and the NOEC obtained for Ceriodaphnia dubia in a reproduction inhibition test (8 days) was also 0.1 ppm. For the secondary consumer Hydra attenuata, the NOEC obtained was 1 ppm in a reproduction inhibition test (7 days). For planarian and fish, only acute tests were performed. For Girardia tigrina newborns the EC50 (96h) was 80 ppm and for fish (Danio rerio, larvaes, 96h), it was >50 ppm. This dye also showed mutagenic activity in the Salmonella/microsome assay but did not present estrogenicity in a bioluminescent yeast estrogen assay. Based on these data we propose a PNEC for the main dye (N-Ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline of 0.6 ppb, considering that this compound would be responsible for the toxicity of the commercial product. This value was based on the lowest NOEC 0.1 ppm (chronic assay P. subcapitata and C. dubia) divided by an assessment factor of 10 because no chronic assay for fish is available and additional 10 because of its mutagenic potential. More studies are being conducted to verify the mutagenic effect of this product in microcrustacea as well as monitoring studies in river waters that receive textile discharges to determine exposure concentrations.

TU 229
Efficiency of chemical mixtures in the industrial control of biofouling by the Asian clam Corbicula fluminea
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The freshwater bivalve Corbicula fluminea (Müller, 1774), commonly known as the Asian clam, is one of the most invasive species of aquatic systems around the world. In addition to the adverse effects on ecosystems, this pest is well-known for its biofouling activity on freshwater-dependent industries. Developing environmentally-friendly methods to control the Asian clam in the industrial context and thus prevent significant economic losses is a major challenge.

The biocide activity of the cationic polymer polydiallyldimethylammonium chloride (polyDADMAC) against the Asian clam has been previously demonstrated. In this study, binary mixtures containing polyDADMAC and either potassium chloride, the molluscicide niclosamide or the insecticide dimethoate were formulated in an attempt to further increase the susceptibility of the clams to the polymer. The three combinations were tested in standard laboratory bioassays and the mortality results interpreted using an isobologram-based methodology. Through this preliminary study, different types of interactions between the chemicals were explored and the extent to which the performance of a potential combined treatment depends on the chemicals' mode of action was assessed. In general, the combined effect of the compounds was found to be antagonist and, therefore, these mixtures are not promising control tools. In order to understand the antagonist nature of the chemicals, preliminary experiments were conducted to elucidate the mechanism of toxicity of polyDADMAC and its interaction with dimethoate in C. fluminea. This study indicated that polyDADMAC neither affects the ability of osmotic regulation of the cells as suggested by the literature for other surface-active molecules nor interacts with the hydrolytic activity of the enzyme cholinesterase, which is the specific target of dimethoate.

TU 230
Industrial enzymes - an example of the environmental risk assessment of an UVCB (unknown or variable composition, complex reaction products or biological materials)
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Industrial enzymes for technical applications like for example detergent enzymes have to be safety assessed and registered under REACH. Enzymes are categorized as UVCB's because they are produced by fermentation and are thus substances from biological origin with varying content of constituents. The enzyme protein together with the constituents derived from the fermentation process is considered to be the substance. Before enzymes with the same catalytical activity, but produced by different production strains, can be considered to be the same substance from a safety point of view, it is necessary to establish the safety of the production strains including the safety of the other constituents. To assure the safety of the other constituents the enzyme industry uses safe strain lineages (i.e. the production organism and methods of modification are demonstrated to be safe) which have been well established over the years as also indicated by scientific publications. This is a prerequisite for establishment of sameness so further ecotoxicological evaluation can be focused on the enzyme protein itself.
Industrial enzymes are general of low ecotoxicity except for proteolytic enzymes, where some exhibit acute aquatic toxicity. Based on the available ecotoxicity data industrial enzymes can with regard to their ecotoxicological profile be grouped in proteolytic and non-proteolytic enzymes. Within these two groups read-across can be applied. Enzymes are readily biodegradable to peptides and amino acids, then further to environmentally harmless carbon dioxide and water as degradation products. Additionally, for the majority of ecotoxicity tests required for REACH registration data waiving can be applied including long-term aquatic toxicity testing and toxicity to terrestric organisms. These tests are considered not relevant due to the fast biodegradation of enzymes, the low absorption and bioaccumulation potential and because enzymes are primarily active in aquatic environments. Thus only short-term aquatic toxicity tests are considered relevant for industrial enzymes.
Based on the current knowledge on industrial enzymes and their intrinsic properties future ecotoxicological testing of enzymes may be avoided by applying read-across and the data waiving approach.
In conclusion, environmental risk assessment of UVCB's like enzymes requires an alternative approach compared to the risk assessment of 'classical' chemicals or well defined mixtures due to the biological origin.

TU 231
A partial least squares based integrated addition model for estimating mixture toxicity
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Studies on mixture toxicity among chemicals find that mixture components at levels below no-observed-effect concentrations (NOECs) may elicit toxicity resulting from the joint effects among the substances. However, chemical risk assessment frequently focuses on individual chemical substances, although most living organisms are substantially exposed to chemical mixtures rather than single substances.
The concepts of additive toxicity, concentration addition (CA) and independent action (IA) models, are often applied to predict the mixture toxicity of similarly- and dissimilarly acting chemicals, respectively. However, living organisms and the environments are exposed to both types of chemicals at the same time and space. Therefore, from the scientific perspective, it still needs to develop an integrated model to predict mixture toxicity from different chemicals practically, regardless of whether mixture components produce similar, dissimilar, or both similar and dissimilar modes of toxic actions.

The objectives of this study are to develop and evaluate a partial least squares-based integrated addition model (PLS-IAM) for not only to overcome the multicollinearity problem which can be occurred between the two independent variables, CA and IA, but also to combine them into the integrated addition model by using the latent variable. In this study, the PLS-IAM was validated by different datasets on mixture toxicity. The results showed that the prediction capability of the PLS-IAM outperformed reference models, the CA, IA, and IAM based on ordinary least squares.

LC04P - Latest developments in uncertainty management – adding value to LCA studies

TU 232
Using sensitivity analysis in developing a characterization model for noise impacts
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Pilkey and Pilkey-Jarvis [1] claim the "virtual impossibility of accurate quantitative modelling to predict the outcome of natural processes on the Earth's surface", due to the inability of environmental model makers to consider all possible sources of uncertainties in their models or due to their unrealistic or even false assumptions.
In Life cycle assessment (LCA) we do use models whose outcome is always affected by a certain level of variability and uncertainty. The numerical solution of these models is, in fact, itself a source of uncertainty [2]. LCA studies often fail to communicate to what extent models, parameters, inventory data and results are influenced by uncertainty [3]. Practitioners and developers often communicate single values for model parameters and characterization factors, negatively impacting on the transparency, usability and trustworthiness of the throughput of the models.
The use of LCA for the support of public decisions has contributed to the increased attention to the quality of data reported by LCA studies. In many cases the result of an assessment used by a policy maker can make a difference to the quality of life and health of (animal or human) populations living at a certain location. In the context of the study of noise, an underestimation error could, for instance, prompt a local authority to cease investing in systems which could prevent the propagation and impact of noise. We proposed a new approach [4] that allows for the characterization of any noise-emitting source and its impact on humans, creating the necessary theoretical structure to accommodate noise in LCA and possibly also make it available for policy advice. Our follow up research aims at testing, analysing and further developing the noise characterization model applying sensitivity analysis (SA) techniques to demonstrate how uncertainty from the input of the model propagates to the output and how each single variable affects the overall variance of the output.

TU 233
Quantifying and propagating uncertainty in regionalized impact assessment: the relevance of spatial aggregation
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Most LCIA methods do not provide uncertainty information to be coupled with uncertainty of LCI. Furthermore, regionalized differences are often not properly addressed, leading to additional uncertainty due to aggregation of spatial explicit characterization factors (CF) on regional or global levels. This lack is a main challenge for practitioners applying proper LCA including uncertainty and sensitivity analyses. On the other hand, concepts for reporting spatial uncertainty by method developers are still under development. In this study, different types of uncertainty for water consumption impacts are analyzed and combined on the endpoint level, showing how uncertainty information can be added to spatially explicit CFs.
In current Life Cycle Impact Assessment, human health impacts due to water consumption have only been addressed by two published methods so far. Both methods assess impacts as DALY (Disability Adjusted Life Years lost) due to lack of water for human use, but the modeling approach and considered impact pathways differ and cannot be used for model scope uncertainty quantification. The method of Pfister et al. (2009) was used as a starting point. Parameter uncertainties were estimated based on analysis of original data sources and, where applicable, on quantitative assessment of the model uncertainty of the data provided by third parties. Spatial variability was also considered when using aggregation on country level. The uncertainties were propagated within the cause-effect impact model by applying the stochastic procedure of Latin Hypercube with the software @Risk.
The average k-values (dispersion factor denoting the 95% confidence interval if the median is divided (lower bound) and multiplied (upper bound) by k) was equal to k=2.76 on the midpoint level and 18.1 on the endpoint level. The aggregation from watershed to country level resulted in an average uncertainty of k=19.2 for the endpoint characterization factors and represents the variability of watershed factors within the country. The results show high spatial diversity of k-values and make it difficult to derive generic estimates of uncertainty, especially regarding the endpoint CFs. However, they can be used to produce maps of uncertainties and also to indicate in which regions

country level data might not considerably add to uncertainty. Such information helps to improve efficiency when dealing with regionalized LCIA. Overall spatial aggregation added considerable uncertainties.

TU 234

Sensitivity analysis for archetypes development in impact assessment of chemicals

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Emission of chemicals is increasing over years and the related impacts are greatly influenced by spatial differentiation. Chemicals are usually emitted locally but, due to persistence and physical chemicals properties, may exert global impact. Variability of environmental parameters may affect the fate and the exposure up to orders of magnitude of difference. Accounting for spatial differentiation of chemical impacts requires the use of multimedia models, at various levels of complexity (from simple box model to computational intense and high spatial resolution model).Trade-off between complexity of high detailed models and global applicability requires the development of suitable archetypes.
In the present study, sensitivity analysis techniques have been applied to MAPPE (Multimedia assessment of pollutant pathways in the environment) to build archetypes of emissions and fate. MAPPE is an advanced, multimedia, spatially resolved (1x1 degree) model, that allows the evaluation of chemicals removal rates from air, water and soil. We evaluate the relative influence of substance properties and of environmental characteristics on the variability in the environmental fate. In particular, using a Montecarlo framework, we applied variance-based sensitivity analysis techniques to find out those environmental parameters explaining the highest share of the variability (namely the variance) in the model outputs. This information is crucial in order to define the environmental dimensions to be used for the definition of the archetypes of emissions and fate, accounting for major differences in the potential impact of the different pollutants.
We present the result of the analysis for the pesticide Lindane. The overall variability of the removal rate from air is up to 5 orders of magnitude, mainly driven by four out of the eleven environmental parameters proposed in the model. These four parameters were then used as basis for building archetypes.

TU 235

Dealing with uncertainties in UNEP SETAC toxicity model

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In the field of LCA, the issue of uncertainty generates a growing interest. Although this issue has not yet been systematically addressed in existing databases and models, it neither can be neglected, nor addressed too simply. Although uncertainty has partly been quantified in USEtox model, several questions remain open to date.
A significant share of this uncertainty arises in model itself, based on the relationship between the data and the intended reality being modeled.
As regular users of the USEtox model, we identify the following sources of uncertainties:
- substance for which no data is available, or missing input parameter value
- parameters for which only an inappropriate or inaccurate value is available
- data for which more than one value is available.(multiple sources and conflicting information)

Given the relative complexity of the mathematical model, uncertainty propagation has been performed using a numerical method.
Well known monte-carlo simulation has been used to take into account the potential variability of the input parameters (substance data and landscape model).
This approach allows us to describe statistical properties of a characterization factor value, based on input data uncertainties. It can also be used to study parameters sensibility.
Taking into account the fact that uncertainty in USEtox database cannot be fully captured within the input parameters variability, other approaches have been studied, which aims at reducing uncertainty.
In approaches to reduce the uncertainty, database documentation, substance coverage, as well as user interaction are key issues.
The determination of the true value for a parameter requires research, experiments and therefore time. Involving stakeholders, discussing and finally deciding on a consensus, so has the current model been built, so the database should be.
Possibilities must be given to each user to view, add, or revise the documentation of a specific parameter. Reporting an error in a parameter value also have to be easy, as well as the possibility to discuss validity of each parameter value.
Our practical experience and ideas, related to management of these uncertainties in USEtox model will be presented .
Those approaches have been implemented in a web application prototype that allows uncertainty calculation and documentation completion.

TU 236

Standard information modules as basis for LCA reliability in the manufacturing context

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In such work the standard-information-modules approach is presented as methodology to reduce uncertainty in the Life Cycle Assessment. In particular such approach aims to overcome common barriers in the modelling of complex systems within the manufacturing context. An application is illustrated with reference to the uncertainty characterization of environmental profiles referred to energy flows. The use of standard modules for environmental profile characterization seems to improve the comparison of different studies by polarizing the improvement analysis on key life-cycle stages.

TU 237

Taking into account variance of primary data in decision making: a method for the building sector

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Life Cycle Assessment (LCA) can be a powerful tool to drive society toward sustainability if used for help to decision. Nevertheless decision step comes often early in the design process where very few data is available. To overcome this issue analysts are forced to use poor quality data or generic data even for foreground system description which is hurtful to results liability and could mislead the decision-maker. In this paper we focus particularly on construction sector which represents about 40% of the total energy consumed in Europe [1]. In the building field, help to decision comes at the planning phase. At this step, only rough data are available but still the planning manager has to choose the best environmental solution regarding its expectations among several building team proposals. The method presented in this paper allows decision-makers choosing between solutions taking into account variance of primary data. It is tailor made to building sector.

In the first step of the method, selection of key parameters is made taking into account both sensitivity of parameter on the results and variance of these parameters. Then standard deviation is propagated using tailor series. This method has been implemented in a real case where decision needed to be made between three building projects. The case study has shown that the method presented in this paper is easy to implement and suitable for complex products as buildings which are tough to describe completely. In some case, it is possible to identify the best building solution even with up to 50% standard deviation on some primary data.

TU 238

Uncertainty evaluation of LCA models input data using Monte Carlo Method

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This is a contribution to the development of methodologies in considering uncertainties in LCA studies. The main results are a solid methodology for dealing with uncertainties in LCA and two stochastic LCA models that enable practitioners to validate their results. The case study consists of an acoustic panel made up of cotton fibers, polyethylene and scraps of jeans. Its LCA model is developed with SimaPro® software. The Monte Carlo tool available in this software is used in order to develop the Monte Carlo Process model. Another model, called Monte Carlo Inventory model, is developed in Matlab® codes, with the model inventory table and in the CML 2 baseline 2000 impact assessment tables, all exported from SimaPro®. The same steps are followed for both models. First, a sensitivity analysis is made in the deterministic LCA model. Next the random parameters are chosen and modeled as random variables associated to a probability density function. Next, the Monte Carlo Method is applied and the convergence is observed. Finally, the results are analyzed. Both approaches are compared in order to observe if the method based on the inventory has the same performance of the method based on the input data. The objective is to obtain a basis of comparison for the stochastic LCA model and the focus is on the methodology development.

TU 239

How certain can we be about LCA after 20 years of harmonisation and standardisation? A critical comparison of methodology and results from ten LCAs comparing disposable cups

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Harmonisation and standardisation of LCA-methodology started in the 1990ties. It happened more than once in this period that contradictory LCA-results were released for a same product (particularly packaging materials). This resulted in intensive scientific debate, and also heated societal debate, especially where product claims were involved. Differences in LCA-results at that time could often be traced back to different decisions and assumption related to product system boundaries, functional unit definitions, technology choices, data sets etc.
In the last two decades LCA-methodology development has made immense progress. We meanwhile have a solid LCA frameworks. ISO 14044 describes the procedures to be followed, and specifies the assumptions and decisions to be made in LCA. Comprehensive and detailed guidelines are provided by national handbooks that still show few differences, but far greater overlap in methodology. With this in mind it is interesting to evaluate whether the progress in harmonisation LCA-methodology has also resulted in harmonisation of LCA-results. For that purpose we evaluated 10 LCA studies, each comparing a range of different types of disposable cups for hot and cold drinks. We

first compared whether they arrived at similar rankings for similar cups, and next compared and evaluated the studies on a large number of methodological issues that are the source for different types of uncertainties. This presentation will present the main results and the surprising conclusions from our study.

EC05P - Occurrence, fate and impact of atmospheric pollutants on environmental and human health

TU 241

Urban background levels of novel brominated flame retardants in ambient air in Southern Bavaria, Germany

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After the technical penta- and octabrominated diphenyl ether mixtures (PBDE) were banned in the European Union in 2004 and decabrominated diphenyl ether is not any longer permitted in electrical and electronic equipment since 2008, there is an increasing demand of 'novel' brominated flame retardants. At present, little is known about the concentrations of these substances in ambient air. Therefore, ambient air was collected between March and September 2011 at the urban background air monitoring station on the premises of the Bavarian Environment Agency, Augsburg, Southern Germany. A sample volume of 960 - 3900 m^3 was collected over a period of 35 days using cartridges with a glass fibre filter and two polyurethane foam plugs. Two low volume samplers were run in parallel. 1,2-bis[2,4,6-tribromophenoxy]ethane (BTBPE), hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), pentabromotoluene (PBT), 2,3,5,6-tetrabromo-p-xylene (p-TBX) and decabromodiphenylethane (DBDPE) were extracted with n-hexane/acetone (1:1, v/v) using accelerated solvent extraction (ASE). The analytes were separated by fractionation on a sulfuric acid-silica column. Purification of the two fractions containing BTBPE and the other flame retardants, respectively, was done on a Florisil®-column and on basic alumina, respectively. After clean-up, samples were analysed by GC-EI-MS. ^{13}C-labeled internal standards were used for quantification. Response factors were calculated using a three-point-calibration. Concentrations varied from 0.08 to 0.34 pg m^{-3} (BTBPE), 0.22 to 0.74 pg m^{-3} (HBB), 0.04 to 0.30 pg m^{-3} (PBEB), 0.42 to 1.3 pg m^{-3} (PBT), 0.15 to 0.23 pg m^{-3} (p-TBX) and 1.5 to 3.6 pg m^{-3} (DBDPE). For the purpose of comparison hexabromocyclododecane (sum of α-, β-, γ-HBCD), polybrominated diphenyl ethers (sum of 7 congeners BDE 28, 47, 99, 100, 153, 154, 183) and BDE 209 were also determined. Concentrations ranged from 3.7 to 12 pg m^{-3} (HBCD), 0.72 to 1.5 pg m^{-3} (sum of 7 PBDE congeners) and 0.45 to 9.5 pg m^{-3} (BDE 209). The financial support of the Bavarian State Ministry of the Environment and Public Health (project no. 7403) is gratefully acknowledged.

TU 242

Halogenated flame retardants in the marine atmosphere from Southeast Asia towards Antarctica

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Halogenated flame retardants (HFRs), which are applied to reduce the inflammability of various consumer products have been industrial chemicals of growing worldwide environmental and political concern in the 1990s and 2000s. Polybrominated diphenyl ethers (PBDEs) were the most applied brominated flame retardants (BFRs) in the 1990s and early 2000 which are known to be harmful. Due to their ongoing banishment since the early 2000s, the industry shifts towards alternative non-regulated non-PBDE BFRs. Similar to PBDEs, most of them are semi-volatile and highly persistent leading to a high potential to be transported over the global oceans towards the Polar Regions.
In the present study the occurrence, distribution and transport of 10 BDE congeners, 8 non-PBDE BFRs as well as 6 Dechlorane compounds in the atmosphere over the Indian Ocean was studied. High volume air samples were taken during a Polar expedition onboard the R/V Snow Dragon from the East Indian Archipelago towards Western Australia and further to Antarctica in 2010/11. Samples were Soxhlet extracted and analysed via GC-ECNCI-MS/MS.
PBDEs were detected in concentrations from 0.08 to 5.1 pg/m^3 with BDE-47 and BDE-209 being the predominating congeners. Among the alternative BFRs, pentabromobenzene (PBBz), pentabromotoluene (PBT), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), hexabromobenzene (HBB), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB) and bis-(2-ethylhexyl)-tetrabromophthalate (TBPH) were detected. HBB showed the highest concentration ranging from 0.12 to 26 pg/m^3, and PBT and PBBz ranged from not detected (n.d.) to 2.8 pg/m^3 and from n.d. to 4.3 pg/m^3, respectively. Dechlorane Plus (DP) ranged 0.23 to 11 pg/m^3 while other Dechloranes remained <0.5 pg/m^3. On a spatial scale, the highest concentrations, especially for HBB, PBT and PBBz, were observed over the East Indian Archipelago showing South East Asia to be an important source of alternative BFRs. The approach of Clausius-Clapeyron Plots clearly indicates that the distribution and transport of PBDEs is dominated by long-range transport with little influence of fresh emissions whereas alternative BFRs, especially HBB, are subject to local sources. We present the first data on occurrence, distribution and transport of alternative BFRs and DP over the Indian Ocean.

TU 243

Contamination of the marine biological reserve of atol das Rocas (Brazil) by persistent organic pollutants

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The Biological Reserve of Atol das Rocas shelters the largest seabird population of Brazil and despite be considered a remote area due to its distance from the mainland, this area is not exempt from the influence of anthropogenic agents, such as persistent organic pollutants (POP) that even has caused the decline of populations of several species of seabirds over the globe, due to bioaccumulative and biomagnified effects on typical top predators. This proposal aimed to determine the occurrence and concentrations of those organic contaminants in respective avifauna. One expedition was performed in February, 2010, where 16 liver samples of two species: Anous stolidus (n=14) and Onychoprion fuscatus (n=2) were collected. Samples were analyzed for organochlorine pesticides (OCPs), PCBs and PBDEs. Briefly, samples were extracted in a Soxhlet apparatus, followed by classic adsorption column and GPC column as clean-up stages. OCPs and PCBs/PBDES were respectively analysed by gas chromatography with electron capture detection and mass spectrometry. Preliminary data showed that PCBs were present in all samples, ranging, in wet weight, from 5.23 - 21.7 ng g^{-1} for the O. fuscatus species and 20.5-27.9 ng g^{-1} for the A. stolidus species with predominance of lighters congeners (tri-, tetra- and pentachlorinated groups). The p,p'-DDE occurred in 88% of the samples of O. fuscatus and in the two samples (100%) of A. stolidus with respective values ranging from <0.19-2.45 ng g^{-1} and 0.50-3.15 ng g^{-1}. HCB was found in 40% of samples of O. fuscatus, with a mean value of 0.35 ng g^{-1} and in the adult individual of A. stolidus in value of 0.65 ng g^{-1}. PBDE 47, at concentration of 4.70 ng g^{-1}, was found only in the sample of adult individual of A. stolidus. The low levels of contaminants suggest a relative degree of isolation and preservation, however the biggest levels seen on the majority of the pollutants groups on adult individual of A. stolidus could also reflect the bioaccumulative and biomagnified trends. The occurrence and distribution profiles of PCBs supports the hypothesis that the main source of contamination in remote areas is due the long range transport, demonstrating the ubiquity of those pollutants on environment. The ongoing samples and analysis of different species may also assist in understanding of the distribution and in the degree of exposure of POPs in remote environments, such as Biological Reserve of Atol das Rocas.

TU 244

Seasonal variation of specific toxic effects of organic air pollutants from year-long sampling campaign

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Air pollutants were described to produce many toxic effects including endocrine disruption. Evaluation of such types of effect cannot be based only on data from chemical analyses. That shows the importance of incorporation of specific bioassays into evaluation part of the air pollution monitoring programs. The utility of these methods in monitoring of atmospheric pollution levels has been shown previously. It is known that levels of pollutants in atmosphere are closely related to type of pollution source and a season period. In our study, we focused mainly on the latter factor. Air samples were collected for one year at a traffic-burdened urban site in Brno (Czech Republic) and a village site about 8 km from Brno city to address the influence of city agglomeration as an air pollution source on the surrounding area. The samples were analyzed for a wide range of parameters and pollutants. In this work, we show the seasonal variation of aryl hydrocarbon receptor (AhR) mediated effects, anti-/estrogenicity and anti-/androgenicity. These parameters were assessed using in vitro reporter gen assays based on mammalian cell lines. This research was supported by GACR P503/10/P249 and CETOCOEN (CZ.1.05/2.1.00/01.0001).

TU 245

Profiles and cold trapping of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in British Columbian mountain soils

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Soils collected at several elevations on five mountains on and near the Northern Pacific Coast of North America were analyzed for 15 Polycyclic Aromatic Hydrocarbons (PAHs) and 54 Polychlorinated Biphenyls (PCBs). Mount Anderson (MA) is located on the West side of Vancouver Island; Grouse Mountain (GM) is in the city of North Vancouver; Four Brothers Mountain (FB) is 100km further inland in the Fraser Valley; Blue Grouse Mountain (BG) is in a fruit growing region 200km northwest of FB by the city of Kelowna; the Chilkoot Trail (CT) crosses from the Alaskan Panhandle into northern British Columbia. MA, GM, FB and the ocean side of CT receive copious seasonal precipitation, while BG and the East side of CT are in rain shadows. PAH profiles were similar across soils, with nearly all analytes found in all soils. Principle component analysis was unable to clearly distinguish between mountains, but suggested the soils collected at the summits of mountains are notably different in their PAH profiles. Total PAH concentrations - by dry weight of soil - were an order of magnitude higher in GM soils than the others. PCBs were present in all soils, but the congener profiles varied dramatically between mountains. Many congeners were below the method detection limits at all sites. GM soils had the greatest number of congeners present with 51, FB had 31 congeners and MA had 24 congeners while only 12 and 6 congeners appeared above method detection limits in CT and BG samples, respectively. Mean total PCBs were highest in the GM soils followed by FB, then MA and lowest in the soils of BG and CT. Thus, where precipitation was the same, proximity to sources appears to dictate PCB levels, but where wet deposition is nearly absent, soils have low levels of PCBs. Despite the low precipitation rate the two easternmost samples from CT had relatively high levels of PCBs; the small settlement and train in that area may be a PCB source. After normalization to organic carbon the concentrations of a few congeners exhibited a positive relationship with elevation, indicating orographic cold-trapping, but only for GM soils. Concentrations of two congeners with quite different physicochemical properties - PCBs 42 and 199 - exhibited fairly strong relationships with elevation ($r^2 = 0.94$, $p < 0.01$, and $r^2 = 0.97$, $p < 0.01$ respectively). The generally low PCB concentrations in the soils of remote mountains may mask the phenomenon of cold-trapping.

TU 246

Recommendations for the use of airsheds for interpreting semi-volatile organic compounds in passively sampled air

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To aid in the interpretation of measurements of Semi-Volatile Organic Contaminants (SVOCs) it is common to use Back-Trajectories (BTs), a calculated approximation of the spatial history of the air sampled. The endpoints of each segment of the BTs can be binned into a grid of cells to create an airshed, which indicates the fraction of the sampled air that passed through each cell. Here, to avoid the so-called 'pole-problem,' a 'geodesic' grid of nearly equidistant cells was employed.

The variability of airsheds in time, both year-to-year and season-to-season was calculated as the sum of absolute differences in endpoint counts in each cell from one time to another. For 24 locations for which BTs were available for 2 or more years inter-annual differences ranged from 9% to 21%, and season-to-season differences ranged from 17% to 53%. For one location for which 13 years of BTs were on hand the differences between the first year and subsequent years was seen to increase with time ($r^2 = 0.69$, $p < 0.01$).

To determine a suitable constraint on departure height of endpoints when assigning potential sources a 7 cell departure area was defined as the 'emitting' cell and the six cells adjacent to it. Using forward trajectories it was determined that after four time steps ~87% of trajectory endpoints were outside of the departure area - this includes points from trajectories that double-back into the departure area. At the fourth time step the 95% percentile of height was ~700m. Thus endpoints that are within the departure area but have a height greater than 700m very likely do not represent air masses that received contaminant from emissions in that cell.

Lastly a significance criterion was derived by assuming that when assigning potential source regions to a measurement only departures from a perfectly 'round' airshed - one in which air arrives at the sampler in equal amounts from all directions - are of interest. Based on this a test criterion is proposed, specific to the temporal resolution of the BT endpoints, to determine the significance of any cells in an airshed. It is recommended that airsheds be generated specifically for the deployment time and location of a sampler, that endpoints with heights greater than 700m be removed from the set and that cells failing to meet the test criterion for significance be ignored when assigning potential source areas.

TU 247

The spatial distribution and potential sources of polycyclic aromatic hydrocarbons (PAHs) over the Asian marginal Seas and the Indian and Atlantic Oceans

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Gaseous and particle-bound polycyclic aromatic hydrocarbons (PAHs) were analyzed in air samples taken on an expedition of the Scholar Ship from January 16th to March 14th, 2008. Samples were taken from the East and South China Seas and the Indian and Atlantic Oceans, providing an opportunity to assess spatial trends and potential sources of atmospheric PAHs over those oceans. Continental sources were still the major reason for the high concentrations of PAHs measured over the oceans. The Σ15PAHs in the gaseous phase were elevated on the approach to China and India, while the highest Σ15PAHs in the particulate phase were found at Chennai Harbor and close to Guinea. The high proportion of fluorene over the East and South China Sea could be a marker of coal and coke related combustion emission from Mainland China. The elevated high-molecular-weight PAHs in particles close to Guinea might be related to biomass burning in Africa. These results are consistent with previous PAH emission inventories and highlight the potential impact of continental PAH sources in China, India and Africa on the global marine atmosphere.

TU 248

PAHs along a mountain forest and clearing: the effect of canopy

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1. Introduction

Forest is regarded as a global sink of semi-volatile organic contaminates (SOCs). The so called "Forest filter effect (FFE)"may be an important driver that transfers SOCs from the atmosphere into the forest system (1). Previous study has focused on the atmospheric deposition and atmospheric-surface exchange of SOCs alone the mountain forest and clearing (2-4). This study aimed to compare atmospheric concentration of PAHs under the forest canopy and nearby clearing the Lys Valley, Aosta, Italy, on the slopes of Mont Mars.

2. Materials and methods

Passive atmospheric sampling (PAS) was performed both in the forest and clearing sites at 700 m, 1000 m, 1400 m, 1800 m, and 2400 m (a.s.l) alone the Lys Valley, Aosta, Italy, on the slopes of Mont Mars. 2 high-volume air samplers were used to collect air samples at 1400m for 4 sampling periods.

3. Results and discussion

3.1. Concentrations

The average value of the total PAHs (ΣPAHs) concentrations of gaseous and particle phase was 1860 and 176.1 pg m^{-3} for the forest, and 1230 and 123 pg m^{-3} for the clearing. Seasonality, the PAH concentrations in the mountain atmospheric decrease dramatically from R1 to R4 at a factor of 3800 for gaseous phase and 5.3 for particle phase, respectively, which indicated that the forest filter effect (FFE) was much higher for particle associate PAHs than gaseous phased PAHs.

3.2. Altitudinal Distribution

PAH amounts in the PAS samples (ng PAS^{-1}) were generally declining with the increasing of altitude alone the slopes of Mont Mars. The declining rate for the forest was much lower than that at the clearing, Take round 1 for an example, the rate was 0.003 for forest and 0.006 ng PAS^{-1} m^{-1} for clearing, respectively. This may due to strong forest filter effect at lower sites where corresponding to high PAH concentrations in the atmosphere.

3.3. Effect of canopy

Most of the F/C values in the atmospheric were >1 from active samples, however, F/C values were generally <1 in the PAS samples, which could responsible for a lower sampling rate of PAS in the forest because of lower wind speed. As shown in Figure 2, log F/C and log KOA showed different correlations in the gaseous (green line) and particle phases (black line).

4. Conclusion

The filter effect of canopy was observed alone the Mont Mars and higher factors was related to low altitudinal sites and atmospheric particle. K_{OA} values can influence the effect of forest canopy.

TU 249

Size-specific particle-gas distribution of the atmospheric polyaromatic hydrocarbons (PAHs) on the spatial and temporal scales

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Although polycyclic aromatic hydrocarbons (PAHs) are usually not included amongst the group of compounds defined as 'persistent organic pollutants (POPs)' due to their higher reactivity, they are a subject to long-range atmospheric transport (LRAT) and can pose significant health risks. As such, they are among the pollutants most commonly monitored in the atmosphere.

The objective of this study was to examine the temporal variability of the size-specific particle-gas distribution of atmospheric PAHs in order to understand the physical and chemical parameters affecting the gas-particle partitioning and consequently the ambient concentrations and fate of selected PAHs.

Atmospheric samples from urban and rural sites in the Czech Republic were collected between October 2009 and October 2010 using a high volume air sampler equipped with a cascade impactor (separating particles into 6 size fractions: < 0,49 μm; 0,49-0,95; 0,95-1,5; 1,5-3,0; 3,0-7,2; 7,2-10 μm). All filters and polyurethane foam (PUF) plugs collecting the gas phase were exchanged weekly, and the individual samples of each fraction were combined into 4 composite seasonal samples.. PAHs in gas and particulate fraction were determined using automatic solvent extraction, silica-gel clean-up and fractionation techniques and GC-MS instrumental analysis.

Interpretation of the data suggests that proximity to primary sources drives PAH concentrations as the concentrations measured in the urban site were significantly higher than those measured in the rural site. Seasonality of concentrations levels (higher winter than summer values) as well as the gas-particle distribution (more compounds associated with particles in winter) was well pronounced at both sites for most of selected chemicals. It has been shown before that PAHs are mostly associated with the finest fraction of atmospheric particles, however, seasonel variability of this size-specific distribution is presented for the first time here.

TU 250

Size-specific particle-gas distribution of the atmospheric POPs on the spatial and temporal scales

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A presence of the particulate matter in ambient air (especially of the particles with less than 10 μm in diameter) has a negative impact on human health as it can induce various respiratory diseases. The fine and ultrafine particles are of special concern as they can penetrate the lungs to the greatest extent and even enter blood stream. Another factor that has to be considered when assessing human respiratory risks are persistent chemicals that are associated with the atmospheric particles and represent additional risk when released in human bodies. Size-specific distribution of these chemicals is an important parameter determining their behavior, fate and effects. Size-specific partitioning of organic chemicals between various fractions of the atmospheric particles has been previously studied for polyaromatic hydrocarbons and it has been demonstrated that they are mostly associated with the fine and ultrafine fraction. However, there is not much known about other groups of compounds, especially emerging pollutants. As such distribution affects the fate of compounds not only from the toxicological point of view but also from the point of the long-range transport of pollutants to the pristine environments, new data are desperately needed.

This paper reports on seasonal and spatial variability of size-specific particle-gas partitioning behavior of selected groups of legacy (dioxins and furans, pesticides) and emerging (brominated and fluorinated) pollutants. Samples have been collected from the urban and rural sites in the Czech Republic for the period of one year (October 2009 - October 2010) using a high volume air sampler equipped with a six-stage (< 0,49 µm; 0,49-0,95; 0,95-1,5; 1,5-3,0; 3,0-7,2; 7,2-10 µm) cascade impactor collecting particulate phase and polyurethane foam (PUF) plugs collecting the gas phase.

TU 251

Potential input of organic pollutants to the Mar Menor lagoon: estimation of seasonal air concentrations using passive and active air samplers

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A significant fraction of organic pollutants access directly to air and can be transported far from their sources associated to particulate material or dissolved. Pesticides are widely applied for agricultural treatments and polycyclic aromatic hydrocarbons are formed as consequence of urban, transport and industrial activities. Mar Menor lagoon is the ending point of drainages from a large agricultural area where a variety of pesticides are used. This lagoon is surrounded by several villages and that have, especially in summer, an intense touristic activity. There is also a military airport close to the lagoon and this area is influenced by a very industrialized pole, which is located at 20 km south.

There are not previous data of pesticides and PAHs concentrations in air of in this area. In order to estimate the levels of organic pollutants in air and the potential atmospheric input in Mar Menor lagoon, passive samplers consisting of polyurethane foam (PUF) disks housed in chambers were deployed at six sites around the basin of this lagoon. Replicates at each point and calibration with a DIGITEL active sampler (at one point) equipped with a PUF module are used to improve the confidence of the determinations and to estimate the sampling ratios for the different detected species.

PUF samples have been extracted with hexane using a pressurized liquid extractor. The extract was concentrated and was analyzed gas chromatography with mass spectrometry. The quantification limits were lower than 10 ng/L for the majority of the considered analytes. This method has been validated for more than 80 organic pollutants (pesticides and polycyclic aromatic hydrocarbons) and up to 23 have been identified in PUF samples from active and passive samplers. The range of estimated air concentrations is very wide (10pg/m3-2ng/m3), and depends on the specific compound, as consequence of the proximity of sources and environmental variables.

Seasonal and spatial patterns have been found for different groups of organic pollutants associated to the main local sources in each case. The larger air levels of anthracene, fluorene and phenanthrene were detected in the proximity to the airport, while pesticides have a more homogeneous spatial pattern, with lower air concentrations by the sea side. Also a clear seasonal pattern has been detected in the case of some specific pesticides, such as chlorpyriphos, with higher concentrations in spring and autumn.

TU 252

Biomonitoring of polycyclic aromatic hydrocarbons by pine needles - analytical alternatives and levels in Europe

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The monitoring of pollutants is a crucial step to assess their environmental exposure to organisms. In general, this task is performed in their own habitat (sediments, soil, water or air), but biomonitoring offers the possibility to estimate the multi-route uptake of contaminants. Given its worldwide presence, adequate characteristics and low availability costs, vegetation has been chosen as a valuable matrix since the 1960s, allowing the passive sampling of a wide range of compounds (especially of atmospheric origin). PAHs are no exception since by their hydrophobic and lipophylic nature, they are prone to partition into the waxy layers of plants and trees (mostly on the leaves) or, in the case of the heavier PAHs, to be deposited as particles in the surface. PAHs are widespread carcinogenic and mutagenic contaminants with natural and anthropogenic sources mainly associated to combustion processes.

The complexity of plant matrices makes the separation of the target compounds from their lipidic structure a hard task and led to the development of multiresidue extraction methods in the last 20 years. This means that biomonitoring studies using these matrices demand for reliable extraction and clean-up procedures, which have been suffering continuous development to reach increasingly lower limits of detection associated with sharper chromatographic resolution.

The efficiency and validity of several extraction methodologies (Soxhlet, ultrasonic extraction, microwave-assisted extraction, accelerated solvent extraction, solid-phase microextraction and hollow-fibre liquid-phase microextraction) prior to a complementary clean-up step when needed were studied for 16 PAHs in pine needles. In concomitance, the levels, patterns and sources of contamination were assessed and compared in sampling sites from Portugal, Spain and Greece.

TU 253

Linear and cyclic volatile methyl siloxanes in Swedish air

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The concentrations of linear and cyclic volatile methyl siloxanes (lVMS and cVMS) were determined in air sampled close to the Swedish background monitoring station Aspvreten, some 100 km south of Stockholm. Parallel samples were collected with daily resolution over one month during November 2011. The sampling rate was 16 m3 d-1. The method was based on an existing method for decamethylcyclopentasiloxane (D5). Modifications were made to account for the strong sorption of hexamethylcyclotrisiloxane (D3). The method was shown to be applicable to the lVMS, whereby L3, L4, L5 and L6 were analysed using tetrakis(trimethylsilyl) ester as the surrogate standard. The concentrations were of the order of 3 ng m-3 for D3, 1 ng m-3 for D6, 5 ng m-3 for D4, and 15 ng m-3 for D5. The concentrations of the lVMS were markedly lower, of the order of 0.2 ng m-3 for L3, 0.04 ng m-3 for L6, and 0.02 ng m-3 for L4 and L5. The difference between the concentrations measured in the parallel samples was in almost all cases < 10%. The influence of air mass origin on the temporal variability of the concentrations of the different analytes will be explored.

TU 254

Analysis of cyclic and linear volatile dimethylsiloxanes in aqueous samples and biota

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As a result of their wide use, siloxanes are spread into the environment both via point sources and via atmospheric long range transport and may be found everywhere in the environment. Recent studies have suggested that siloxanes may have direct or indirect toxic effects on various biological processes. Therefore, there is a need of fast, reliable and sensitive methods for the ultratrace analysis of siloxanes in environmental samples. However the routine analysis of these compounds is still challenging because of the low concentration at which they occur (especially in, their high volatility and because of their common occurrence in laboratory instrumentation, including gas chromatography columns, septa and sorbent phases).In this work, different analytical schemes for the analysis of cVMS and lVMS in wastewater and biota is presented. These methods are based on ultrasounds assissted extraction for biota samples, and liquid-liquid extraction for water and wastewater samples followed by gas-chromatography coupled to tandem mass spectrometry (GC-MS/MS). The new analytical methods were validated for their application to the analysis of water, wastewater and biota samples, offering excellent reproducibility, recoveries 63-113% and 40-112% and limits of detection 400 and 1600 pg/g and 0.5 and 7.5 pg/mL in biota and wastewater, respectively. Finally, the occurrence of siloxakes in more than 30 wastewater treatment plants in Catalonia (NE, Spain) was assessed, and the results confirmed the presence of the selected siloxanes at concentrations of ng/L in the final effluents. On the other hand, the analysis of biota samples using an extraction step by ultrasound-assisted extraction was applied for the determination of these compounds in different biota samples from the Llobregat River, the Mediterranean Sea and krill (*Euphasia Superba*) samples from the Antarctic ocean will be presented and discussed.

TU 255

Exchange and partitioning of POPs between the atmosphere-vegetation-soil compartments

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Even though the production and usage of many POPs have been banned decades ago, due to their persistence, they can be still found in the global environment, where in terrestrial environment they cycle between soil, vegetation and atmosphere. POPs can be transferred from the atmosphere to vegetation and soil surfaces by diffusive exchange, dry or wet deposition. A number of studies have shown that POP burdens in vegetation and soil generally reflect spatial trends of concentrations in the air. Vegetation can also be used to identify temporal changes of POP concentration in the air over a range of years whereas bulk soil responds much slower. However, POPs attached to soil/vegetation surfaces may revolatilize delivering POPs to the air, enabling them to be transported in the atmosphere or taken up by organisms at different levels of the food chain. The objective of this work is to assess the role that vegetation plays in the overall air-soil-vegetation partitioning and as a control of POP concentrations in the atmosphere and soils.To study the exchange and partitioning of POPs between soil-vegetation-atmosphere, different sampling campaigns were carried out in temperate (sampling sites at northwest UK) and polar environments (Antarctica Peninsula).Results obtained in this study are close to the those predicted by McLachlan, 1999. Higher concentrations of PCBs, pesticides and PAHs have been found in the air sampled close to vegetation surface in comparison to ambient air concentrations, suggesting that vegetation compartment can also deliver POPs, especially the less volatile ones to the atmosphere, even though soil and vegetation seem to be in close equilibrium. The influence of surface temperature, lipid content in the POPs accumulation and on the magnitude of re-emission were also studied. Results from temperate and polar environments will be compared

TU 256

Endosulfan transport from agricultural fields to Everglades and Biscayne National Parks in South Florida (USA)

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The environmental health decline and the loss of organism diversity of South Florida (USA) ecosystems have been attributed to nutrient inputs from nearby urban and agricultural areas. Intensive agricultural pesticide use may also challenge these ecosystems. One possible mechanism is pesticide release to the atmosphere after application which can be enhanced in this region due to the calcareous soils, frequent rainfall, and high humidity and temperatures. A study was conducted to examine the atmospheric fate of the widely-used insecticide endosulfan. Air samples were collected over a five-year period (2001 to 2006) at a site within the agricultural community of Homestead, Florida and at sites located in nearby Biscayne and Everglades National Parks (NPs). Endosulfan emissions from agricultural areas around Homestead appeared to influence air concentration observations at the NP sites. During an intensive sampling campaign, the highest total endosulfan concentrations at the NP sites were observed on days when air parcels were predicted to move from Homestead towards the sampling locations. The α-endosulfan fraction (α/(α+β)) was used to examine the contribution of pesticide drift versus volatilization to the overall residue level. The formulated product has an α fraction of approximately 0.7, whereas volatilization is predicted to have an α fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift contributes to the atmospheric concentrations. The median fraction at Everglades NP was 0.88 and 1.0 during high and low agricultural activity, respectively, and at Biscayne NP was 1.0 year round indicating air concentrations are primarily influenced by regional volatilization. The near-irreversible isomerization after application of β-endosulfan to α-endosulfan also influences the value of the α fraction.

TU 257
Raman microscopy as a tool to examine agricultural sources of PM10
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Emissions of particulate matter (PM) from animal feeding operations (AFOs) pose a potential threat to the health of humans and livestock and can lead to decreased air quality. Current efforts to characterize PM emissions from AFOs generally examine variations in mass concentration and particle size distributions over time and space, but these methods do not provide information on the sources of the PM captured. Raman microscopy was employed to characterize the distribution of sources present in PM_{10} emitted from a large cattle feedlot. Spectra from potential source materials (dust from unpaved roads, manure from pen surface, and cattle feeds) were processed and compiled to create a spectral library. Principal component analysis followed by cluster analysis was used to classify the source library spectra into specific groups. A multivariate statistical analysis approach was developed to identify the source of particles collected on PM_{10} sample filters. Source characterization results from samples collected at a cattle feedlot over a typical hot summer two-day period indicate that materials from the cattle pen surface contributed more than 50% of the total PM_{10} particles captured followed by unpaved roads at approximately 20% and then feed materials. Results indicate this approach could be used in a number of different agricultural emission characterization scenarios.

TU 258
A langmuir-hinshelwood fit of atmospheric reactions of OH radicals with semivolatile, aerosol-borne compounds in chamber experiments
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A number of semivolatile compounds and proxies of environmental compounds, such as oleic acid, long-chain alkanes, polycyclic aromatic hydrocarbons, plasticizers, PCBs, brominated flame retardants and various pesticides have been investigated for their atmospheric degradation rates by OH radicals and/or ozone. Some of them have been examined in the gas phase and some of them in the adsorbed state as thin films or sub-monolayers on appropriate solid materials in either flow reactors or aerosol chambers, by exposing them to OH radicals or ozone at known levels.
In the last decades, numerous studies were performed to investigate the reaction of OH radicals with compounds in the gas phase. However, due to its complex mechanisms, aerosol-borne reactions with OH radicals are poorly understood. Only recently, the Langmuir-Hinshelwood mechanism and the Eley-Rideal mechanism have been applied on the reaction of semivolatile compounds with O_3, considering different adsorption processes of reactants and the gas-particle equilibrium characteristics.
In this work, we applied both mechanisms in the analysis of the reaction of aerosol-borne terbuthylazine with OH radicals in our simulation glass-smog chamber. Home made silica particles were used as carrier material. Compared to previous <10 nm particles (Aerosil 200), these larger particles (diameter about 160 nm) have less tendency to agglomerate. Compared to previous studies, a negative correlation with OH concentration was found for the second order reaction rate constant. At relatively low OH concentrations, the reaction could be explained by the Eley-Rideal mechanism, while at higher OH concentration, the Langmuir-Hinshelwood mechanism is more appropriate to explain concentration-time dependencies. Because the adsorption could be affected by gas-particle equilibria, results from different experimental setups (e.g. Chamber experiments and flow tube experiments) are assumed to be different.
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TU 259
Analysing PBDE in house dust samples with the TSQ Quantum Ultra XLS GC-MSMS in EI- SRM mode and GC-MS negative chemical ionization in SIM; a comparison of two analyzing techniques
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Polybrominated diphenyl ethers are a class of compounds used as flameretardants in a wide variety of household apparatus and furnitures.
Certain congeners have been banned completely and are currently on the list of the Stockholm convention on persistant organic pollutants. (1,2)
There is an ever growing concern about the health risks being exposed to these class of compounds and one of the sources of human intake is next to nutrition, inhalation of indoor air and house dust.
In this application note, a comparison is made between two analytical techniques; GC-MS-MS and GC-NCI-SIM, evaluating detection limits, repeatability and selectivity in matrix.
A broad range of PBDE was analysed, ranging from tri- up to deca BDE, together with another class of brominated flame retardants such as decabromodiphenylethane (DBDPE), tetrabromethylcyclohexane (TBECH), and 1,2,5,6,9,10-Hexabromocyclododecane (HBCDD).

TU 260
Human exposure to flame retardants in different occupational settings from Pakistan
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Flame retardants (FRs) are widely used in a variety of consumer's products, such as plastics, textile coatings, electrical appliances and printed circuit boards to inhibit the development of fires. Studies have shown that these chemicals can leach out into the environment. In order to investigate the presence of FRs in the specific occupational setting and human exposure to FRs, we collected dust, serum, and urine samples from individuals working at electronics and textile market in Punjab, Pakistan. Polybrominated diphenyl ethers (PBDEs), novel brominated FRs (NBFRs), hexabromocyclododecanes (HBCDs) and organophosphate FRs (OPFRs) were quantified in dust, serum, and urine samples. FRs were extracted from dust, serum, and urine using solid-phase extraction (SPE). In dust and serum extracts, PBDEs and NBFRs, were analysed by gas chromatography (GC) coupled to mass spectrometer (MS) operated in electron capture negative ionization (ECNI), while OPFRs in dust were analysed by GC/MS in electron impact (EI) mode. The separation and determination of α-, β-, and γ-HBCD isomers in dust and serum was achieved using liquid chromatograph (LC) coupled to triple quadrupole MS system operated in the negative electrospray ionization (ESI) mode. OPFR metabolites were analysed in urine using LC-MS/MS in negative ESI mode. Generally, large variations in the individual concentrations were found within and between the investigated groups. The present work indicates that the workers in the studied area are occupationally exposed to various FRs, with probably indoor dust ingestion and air inhalation as a major source of exposure. Correlations were performed between the levels of FRs in dust and serum or between dust and urine samples. For workers, using the 5th, median and 95th percentile concentrations in dust samples different exposure scenarios were calculated via dust ingestion. Exposure scenarios were calculated assuming 100% absorption of intake dust, a 10 hr/day working shift, 70 kg body weight (bw), mean dust ingestion (1.25 mg/hr) and high dust ingestion (3.13 mg/hr). Exposure assessment values were lower than RfD values. Human exposure to FRs seems to originate from a combination of different sources, including indoor sources and diet; however, further studies investigating serum and urine samples from a larger number of occupationally exposed workers are necessary for a more complete assessment of human exposure pathways to these environmental contaminants.

TU 261
Use of volatile organic compounds (VOC) in consumer products and comparison between the European and US reactivity models for assessment
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Use of Volatile Organic Compounds in consumer products and comparision between the European and US reactivity model for assessment:
The environmental impact of Volatile Organic Compounds (VOC´s) is their ability to react with NO_x in the lower atmosphere in the presence of sunlight and heat to produce ground -level ozone, also called summer-smog. Summer conditions favour the formation of ground-level ozone primarily because of increased ultraviolet radiation, temperature and low wind speeds. The reactivity potential of VOC´s in Europe is defined as Photochemical Ozone Creation Potential (POCP); developed by Derwent et al (1998). In USA / California the concept describing the reactivity potential of VOC´s is the Maximum Incremental Reactivity (MIR); developed by Carter et. al (2006).
MIR´s and POCP´s both measure the reactivity towards ozone production of a given VOC. The MIR scale is reported as the mass of additional ozone produced per mass of addition VOC reacted. POCP´s report the additional ppb of ozone produced by an additional emission of that VOC, expressed relative to that produced by the same emision of ethylene as reference.
International consumer companies with global brands have the challenge to meet different aspects of regulatory requirements to reduce their impact of VOC´s. Some examples

are discussed within the poster presentation.

TU 262

Distribution of linear and cyclic volatile methyl siloxanes in indoor air samples and implications for human exposure

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Cyclic (D3, D4, D5, D6) and linear (L2-L5) volatile methyl siloxanes are widely used in personal care products and cosmetics, as well as in industrial applications (biomedical products, surface treatment agents, plasticizers and construction materials). These compounds are of environmental concern due to their volatility, persistence and tendency to bioaccumulate.

The purpose of this study was to investigate their occurrence and distribution in indoor air environments, including domestic (i.e. bathrooms, living rooms, boys/girls rooms) and non domestic (supermarkets, offices, schools, museums) environments. An extensive air sampling campaign was performed contemporarily in the UK and Italy, between May and August 2011. Indoor air samples (n = 100) were collected on adsorption Tenax GR cartridges (60/80 mesh, Markes) using conventional portable air sampling pumps (i.e. GilAir3, Gilian- Sensydine), operating at a flow of about 120 mL min-1. Sampling cartridges were desorbed using the Automatic Thermal Desorber UNITY2 coupled to a GC/MS-system. Concentrations of total siloxanes ranged from 20.6 to 467 ug/m3 and from 55.7 to 424 ug/m3 in Italian and UK samples, respectively. Cyclic volatile methyl siloxanes (in particular D3 and D5) accounted for ~90% of total siloxane concentrations in all samples.

The highest siloxanes concentrations were found in bathrooms; in particular, D5 represented the principal compound probably due to its dominant presence in most of hair-care products, skin lotions, cosmetics, household products and cleaning agents. Personal lifestyles and everyday-life behaviours (e.g., selection of products, frequency of application, amount applied) had a determinant effect on the amount of total siloxanes found in both Italian and UK adult and living rooms. No significantly differences in concentrations were found in other indoor domestic environments. The daily inhalation exposure (DIE) to cyclic and linear volatile methyl siloxanes was also evaluated.

TU 263

Source contribution of atmospheric PAHs at urban and industrial locations in the city of Novi Sad, Serbia

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Data on polycyclic aromatic hydrocarbons (PAHs) in ambient air accessed at urban and industrial locations in the city of Novi Sad, Serbia, have been analyzed in order to determine emission sources and their contributions to the concentrations of particle-bound PAHs. Previous studies have demonstrated that the major contributors of PAHs in urban areas are the emissions from vehicle exhaust, and emissions releases from industrial processes like aluminium production, creosote and wood preservation, waste incineration, cement manufacture, petrochemical and related industries, commercial heat/power production etc. The sampling campaigns have been conducted at three sampling sites, during the two 14-day periods. The first site was situated near industrial area, with a refinery, power plant and heavy-traffic road in the vicinity. The second site was located nearby the heavy traffic area, especially busy during the rush hour. The third site was residential district. Non-heating sampling period lasted from June 26th to July 9th 2008, while sampling of ambient air during the heating season was undertaken from January 22nd to February 4th 2009. 104 air samples were collected using a high volume air sampler TCR Tecora H0649010/ECHO HiVol with quartz fiber filters (QFFs). 16 US EPA polycyclic aromatic hydrocarbons were determined in all samples using a gas chromatographer Shimadzu GC-2014 with a flame ionization detector. The total average concentrations of PAHs ranged from 0.4 to 3.16 ng/m3 during the non-heating period and from 0.05 to 36.81 ng/m3 in the heating period. Various techniques, including diagnostic ratio (DR) and principal component analysis (PCA), have been used to define and evaluate dominant emission sources of PAHs. Diagnostic ratio analysis indicated that vehicles, diesel or/and gasoline, industrial and combustion emissions were potential sources of PAHs in Novi Sad. Additionally, principal component analysis was used to constrain the potential sources. The results show that vehicular emissions are the predominant source of particle-bound PAHs in Novi Sad, Serbia. Less dominant emission of PAHs comes from stationary combustion sources such as home heating during the winter and oil refinery processes during the whole year.

TU 264

Polybrominated diphenyl ethers (PBDEs) and other flame retardants in adult Pipistrelle bats (Pipistrellus spp) from an urban area of NW Britain

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Bat (*Pipistrellus spp*) livers of 30 adult males (M) and 23 adult females (F) from an urban area of the NW Britain, were analysed for 23 PBDEs congeners (Tri to hexa-BDEs) as well as for other flame retardant (pentabromomethylbenzene, pentabromoethylbenzene, hexabromobenzene, hexabromocyclododecane (HBCD) and the two isomers of Dechlorane Plus (DP).

The dominant PBDE congeners were BDEs 47, 99, 100, 153, 154 and 183 in both M and F, which are the same dominant congeners present in the penta technical mixtures. However, bats have a lower proportion of PBDE 47 than the technical mixtures, suggesting degradation of this compound. Total BDEs were dominated by penta-BDEs (65% of the total), followed by hexa-BDEs (with just over 20%) for both males and F.

The sum of the PBDEs analysed showed a wide range (10.4 - 8191 ng g-1 wet weight (ww)) of concentrations, with a mean of 961 ng g-1 ww. Males had on average twice the concentration of PBDEs than F (M: 1182 ng g-1 ww F: 673 ng g-1 ww), although this difference was not statistically significant, possibly a result of the small sample size. Pentabromomethylbenzene was not detected in any sample and pentabromoethylbenzene and hexabromobenzene were only found in a few individuals. In our samples we only could detect the anti isomer of DP, with concentrations between 1.6-38.4 ng g-1 ww. This may have been expected because this is the dominant isomer in the technical mixture. However, we only found DP in M, this may be the result of F offloading accumulated residues to the foetus and to juveniles via milk, as DP is highly lipophilic. The lower residues of PBDEs in F than M is likewise consistent with transfer of residues from mothers to offspring. In contrast however, HBCD (sum of the 3 diastereomeric pairs of enantiomers) was found in higher concentrations in the F (mean= 176.9 ng g-1 ww) than in the M (76.0 ng g-1 ww), and this suggests that there may be sex differences in exposure to or metabolism of this compound. In conclusion, this is the first study that we are aware of to report tissue concentrations of PBDEs and some of their emergent replacement compounds in bats from Europe.

TU 265

Atmospheric pollution due to methane seepage from surface rock layers

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Methane emission contributes to the growing background concentration of ozone, an air pollutant that impacts on air quality and, hence, human health. In particular, methane seepage from surface rock layers into the atmosphere is a problem in many parts of the world. The ground surface is the zone where the processes of weathering are very active. In the presence of gas-bearing rock layers (gas sources) located at a shallow depth below the ground surface, weathering-induced fractures in surface rocks can conduct gas from gas sources into the atmosphere. In the southern part of Donetsk city (Ukraine), weathering-induced fractures in very weak rock (sandy shale) are interconnected and form a system of through channels. The values of gas flow through fractures and intensity of fractures were measured in situ. Observations show that number of fractures per meter measured along traces of through channels is between 34 and 69, and total fracture length of through channels per unit area ranges from 19.5 to 21.6 m/squared meter. It is established that fracture aperture width along trace of through channel can reach 7 mm, and value of the fracture aperture tends to decrease with increasing depth below the ground surface. In situ study shows that role of the system of weathering-induced through channels in the process of gas emission to the atmosphere is much more significant than the role of rock matrix. Indeed, gas flow from through channels is 10.4-19.8 times larger than gas emission from rock matrix, and weathering- induced fractures conduct large portions (91-95 %) of gas emitted from ground surface. The gas flow from individual through channel ranges from 0.00069 to 0.00136 cubic meter/s. The study of impact of methane seepage on human health shows that there are cases of methane poisoning of people living on the ground floor. The frequency (F) of the methane poisonings was statistically analysed. As a result, it is established that frequency F is dependent on average value of gas volumetric flow rate from individual through channel (q), number of through gas-conducting channels (k) located within a 20 m radius around each dwelling house. It is defined that value of F increases according to exponential law, where a variable exponent is the gas flow (q) multiplied by logarithm of the number (k) of gas-conducting channels.

TU 266

Enviromental impact of implementing alternative fuels in a Spanish cement plant

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Cement industry produce around 5% of the whole world anthropogenic CO2 emissions, either as direct (descarboxylation of calcium carbonate -raw material-) or indirect (fuel combustion) emissions. In order to reduce the amount of CO2 emitted by the cement industry, new environmental polices promote the use of alternative fuels such as sewage sludge and refuse derived fuel, among others. Because a reduction of CO2 and costs (fossil fuel saves) may be expected, this activity has resulted to be very environmentally and economically beneficial. On the other hand, the population living closest to the cement plants are generally concerned about the possible effects on the environment and health derived from the use of these alternative fuels. In some cement plants of Catalonia (Spain), the concern of the neighbouring population is especially important, as there is a short distance between cement plant and homes. This short distance is sometimes due to poor (or inexistent) urban planning, illegal construction and the lack of legislation in the past, the distance between populated centres and industrial facilities have been notably shortened.

In Catalonia (Spain), in the last years, the cement industry has started to use different kinds of alternative fuels for partial replacement of the energy, which traditionally comes from fossil fuel. To ensure that this fuel partial substitution will not cause a risk to the environment and health of residents around the cement plant, various environmental monitoring campaigns have been conducted in the surroundings of facilities of different characteristics, in terms of used fuel (sewage sludge, refuse-derived fuel, etc.) or zone characteristics (urban, suburban or rural). In each campaign, several samples of environmental monitors (air herbage and soil) were collected before and after the alternative fuel implementation, and the content of metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) was determined.

The findings showed no significant differences between campaings regardless of the use of alternative fuels. In conclusion, the current results support and encourage the

option of using alternative fuels in cement plants working with the Best Available Technologies (BAT).

TU 267
Should the neighbourhood of cement plants be concerned when alternative fuels are used?
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In recent years, co-combustion of alternative fuels in cement plants has become an increasing practice in many countries. The benefits associated to the replacement of fossil by alternative fuels such as sewage sludge or refuse-derived fuel (RDF), are both economically (fossil fuel saving) and environmentally (e.g., reduction of CO2 emissions, reuse of by-products) evident. These benefits are more important taking into account that cement production is one of the main CO2 emitting manufacture industries, but also due to the important rising of fossil fuel prices. Furthermore, considering the notable consumption of fossil fuels by the cement industries, the European Union is encouraging the enhancement in the amount of alternative fuels used in cement kilns.
In the present work, the result of a 3-year (2008-2011) environmental monitoring campaign around a cement plant located in the metropolitan area of Barcelona (Catalonia, Spain) was presented. The aim of this study was to evaluate the potential changes in the environmental levels metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in the vicinity of a cement plant before and after using sewage sludge as alternative fuel. The concentrations of PCDD/Fs and metals were determined in soil, herbage and air samples around this facility before and after the partial fuel substitution. The potential changes on the health risks for the population living around the facility were also assessed.
In general terms, no statistically significant differences in the pollutant values were found between the 2 scenarios (before and after the partial fuel replacement), with the exception of a few significant decreases in some metals. Besides, the carcinogenic and non-carcinogenic risks due to the exposure to metals and PCDD/Fs were below the national and international safety limits.

TU 268
Environmental impact of mechanical-biological treatment systems. Human health risks of chemical and microbiological pollution
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Due to legislative, environmental, economic, and social limitations, the identification of sustainable disposal pathways for the management of municipal solid waste (MSW) is a very important matter. The mechanical-biological treatment (MBT) approach presents many advantages in comparison to other waste management possibilities. However, adverse health effects related to this practice are not well known, as a varied typology of microbiological, such as bacteria and fungi, and chemical pollutants, such as volatile organic compounds (VOCs), are generated during waste composting and similar processes. Some of these contaminants can be the cause of a variety of infectious diseases, as well as allergies and toxic effects or generating malodorous and hazardous properties. In 2010, a program was initiated to monitor air levels of VOCs and microbiological pollutants near a MBT plant (Ecoparc-2) in Montcada i Reixac, (Barcelona, Catalonia, Spain). To investigate the temporal and seasonal trends of chemical and microbiological pollutants, four 6-monthly campaigns were performed. Air samples were collected at different directions and distances from the facility. The levels of total bacteria ranged from 160 to 1054 cfu/m3 (colony-forming units per cubic meter) with a high increase in the last survey (summer 2011) compared to the initial (winter 2010). In fact, the highest concentrations were detected in the summer campaigns. Fungi at 37ºC were also important in the hot seasons with values of 716 and 106 cfu/m3 (summer 2010 and 2011, respectively) versus 60 and 61 cfu/m3 (winter 2010 and 2011, respectively). The concentration of the remaining microbiological agents (gram-negative bacteria and the more specific Aspergillus fumigatus) was rather low, with a lack of temporal/seasonal differences. On the other hand, the highest mean concentration of VOCs was found in the third campaign (45.7 µg/m3), with values between 4.55 and 120.9 µg/m3. For these chemical compounds, the greatest concentrations were found in two winter campaigns. The current exposure to those chemicals, estimated from the environmental burdens, should not mean additional non-carcinogenic or carcinogenic health risks for the population living nearby. Finally, a fate and transport model was also executed to validate the acquisition of data from outdoors, as well as comparing modelled and experimental results.

TU 269
Environmental risk management in confined spaces. A study case of an archive like a model of indoor environment involved with biodeterioration
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Proper management of specific indoor environments, such as those that preserve Cultural Heritage, cannot fail to take into account the interactions they may have on human health. The Historical Archives, where cellulolytic microfungi and bacteria find a comfortable environment for their development, are of particular interest in this sense.
In fact, one of the major causes of paper degradation is the biodeterioration, which develops with specific environmental conditions. Therefore, it is necessary to conduct research linking aerobiology with environmental Archives, to understand the conservation state of the documents and, also, the possible risks for the Archive's operators and for people who work within these environments.
The study case involves the Contemporary Political History Archives sites in Ca' Tron, Treviso (Italy) in the Veneto countryside. A specific seasonal aerobiological protocol was developed to monitor the presence of microorganisms both in the air and on the documents stored, the temperature, humidity and light conditions, and the detection of dust (PM10 and PM2.5). For the aerobiology analysis, active and passive samplings were performed. Volumetric samplers were used to measure the concentration of microbes in air (expressed by CFU/m3), while Petri dishes with different culture media (Nutrient Agar, Malt Extract Agar and Malt Extract Agar plus Chloramphenicol) were used for qualitative microbiological sampling (expressed by IMA, CFU/dm2/h). In addition, two parameters were considered for the surface contamination, using nitrocellulose membranes: the Microbial Buildup (MB, the total number of microorganisms collected on a surface in 30 second) and the Hourly Microbial Fallout (HMF, the number of microorganisms collected during 1 hour). In this way, it was possible to:
- define the conservative situation of the Archive;
- identify microbial species present both in the air and on the documents and then identify those potentially cellulolytic and those dangerous for human health;
- to propose measures to improve archive's conservation and thus prevent degenerative risk situations, like the Sick Building Syndrome.

TU 270
Persistent organic pollutants (POPs) in ambient air, human breast milk and edible fish - a potential for health risk in Ghana
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Levels of persistent organic pollutants (POPs) in rural and urban background air of southern Ghana were measured in 2008 using polyurethane foam (PUF) disks passive air samplers (PAS) (referred to as PUF-disk samplers). The objective of this study was to quantify the levels of POPs in air and to establish their baseline information for future monitoring and trend studies. This is the first comprehensive study reporting on levels of POPs in background or ambient air in Ghana covering the coastal zone and the middle belt. POPs in pooled and individual human breast milk samples collected from lactating mothers countrywide were also determined using a high-resolution gas chromatography interfaced with a high-resolution mass spectrometer (HRGC-HRMS). This constitutes the first comprehensive nationwide human breast milk study of assessing risks of HCHs for the general population of Ghana. The results of the study showed that the general population of Ghana is widely exposed to POPs although the current levels are generally low. POPs measured in selected edible fish from three freshwater bodies, namely Lake Volta, Lake Bosumtwi and Weija Lake in Ghana shows that there is a potential health risk from POPs for the general population of Ghana because fish is one of their important protein sources.

TU 271
Toxicity screening of fine particles by a novel microbial test battery
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Burning of wood for heating purposes has become extremely popular wordwide due to the increasing costs of fossil fuels. Claimed as renewable energy source, wood has often been ignored as a source for fine particles causing toxic effects. Rapid reproducible and validated biotests for toxicological characterisation of such particles exist neither for cells nor for micro-organisms. Thus there is a need for established microscale toxicity screening of unknown combustion born particles.
Hence a test battery consisting of two ISO standardised bacterial contact assays and one yeast biotest were modified for the use with fine dust. Having different endpoints these tests provide a toxicological "fingerprint". In order to account for bioavailability of contaminants adsorbed on surfaces of fine particles, such were used as a whole not as extracts.
Particulate matter was gained electrostatically from different furnaces in the flue gas channel. Suspended in water, vortexted for 1 min, and ultrasonificated for 15 min samples were applied in a concentration range of 0.001 - 1.0 mg/mL. Particle sizes (5-10 µm) in these suspensions were measured by dynamic light scattering. The control contained no particles, solely deionized water.
Cytotoxicity in the aerobic Arthrobacter globiformis contact assay was determined via dehydrogenase activity (DHA). Genotoxicity was determined via Umu-Test using a genetically modified Salmonella strain. The Yes-Test was employed to determine potential estrogenicity of particle samples.
Contrary to FP A, FP B exhibited cytotoxicity between 0.1 and 1 mg FP/ mL. High contents of Zn supposedly caused cytotoxicity in B. FP A was slightly genotoxic (+S9) at 1 mg/mL and potentially estrogenic. Besides PAHs, other heterocyclic compounds might have caused this result. Further investigations are under construction.
These preliminary results suggest suitability of endpoints determined in the modified tests for risk assessment of fine particles.

TU 272
Airborne particulate matter induced pro-inflammatory effects and oxidative stress in A549 cells
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Clean air is a basic requirement for human health and well-being. An average daily inhalation of 20 m^3 of air is characterized by an exposure to many different pollutants. Apart from the classic gaseous pollutants airborne particulates continue to pose a significant threat to human health worldwide.
The objective of this study was to compare the toxicological effects of different source-related particles in regard to their chemical composition. In this context we investigate airborne PM from different sites in the Region of Aachen. A549 cells were exposed to increasing PM concentrations followed by analyses of cell viability, pro-inflammatory and oxidative stress response.
The results showed a seasonal and location dependant variability of the PM concentration for both particle fractions. Chemical analysis of these particles indicated the presence of 21 elements, water-soluble ions and a multitude of different PAH. The major inorganic components are the crustal elements (Ca, K, Mg, Na) and the (transition) metals Al, Fe, Zn. In contrast to metals and PAH, the concentration of ionic species is only approx. 50 % higher at the rural site, compared to the urban site. Water-soluble particulate matter extracts induced a concentration - and time-dependent decrease in cell viability and an increase in pro-inflammatory and oxidative stress markers. The samples of the urban traffic location, characterized by a high concentration of elemental/organic carbon and metals, induced the highest pro-inflammatory and oxidative activity. Due to the combination of chemical-analytical and toxicological methods a characterization of PM induced cause and effects are possible.
The response of A549 cells to ambient PM was markedly different comparing specific samples from various sampling sites and emission sources in the region of Aachen. Our results support other investigations regarding the importance of the chemical compositions and there PM induced toxicity in vitro.
The study is embedded in the project City2020+ which is part of the interdisciplinary Project House HumTec (Human Technology Center) at RWTH Aachen University funded by the Excellence Initiative of the German federal and state governments through the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG).

TU 273
Selectivity of PCBs at low level with high precision using GC-MS/MS Triple Quadrupole
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The low level analysis of polychlorinated biphenyls at levels required by EPA method 1668 has required the need of a high resolution GC/MS system. Using triple quadrupole GC-MS/MS can achieve the needed detection limits plus offer specificity for confident identification and confirmation. This application will explore the relationship between resolution, selected reaction monitoring (SRM) and speed of acquisition to achieve precise quantification and identification of the chlorinated compounds. The studies were performed in matrix, demonstrating that this isolation can readily remove matrix interference at low target compound concentrations. In addition, this method will demonstrate that the quality control criteria of EPA Method 1668 can be met using a GC/MS triple quadrupole system. Advantages for the laboratory are the reduction in cost of the equipment relative to high-resolution GC-MS systems, along with the ability to use the system for more than just specialized applications. In addition, a library of transitions for the PCBs and other compounds simplifies method development and verification, simplifying the adoption of GC-triple quadrupole MS for this analysis.

TU 274
Analysis of pesticides in water samples based on the combination of solid phase extraction and solid phase microextraction coupled to gas chromatography mass detection
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The increase of the agricultural surface area in Argentina has triggered the generalized use of pesticides, which are spread across soils contaminating both surface and ground-waters. The aim of this study was: (a) develop a sensitive Gas Chromatography tandem Mass Spectrometry (GC-MS) method for the quantification of the most widely used pesticides at environmentally relevant concentrations and (b) test this method with surface waters collected from the Suquía River basin, Córdoba, Argentina.
Sample pretreatment involves a combination of Solid Phase Extraction (SPE) and Solid Phase Microextraction (SPME) affording highly effective enrichment of six pesticides, which can be analyzed at ppb levels. We tested the effect on SPE of cartridges and elution solvents as well as type of fiber, absorption-desorption temperature and time, head-space or immersion for SPME. A fractional factorial design was applied to obtain optimal conditions. GC and MS operational issues were also optimized to afford LODs in the same range that ECD detectors. The optimized method was validated in terms of linearity, precision, recovery and repeatability, showing that the proposed procedure is sensitive (LODs ranging 0.2 to 3.5 ng L'1), precise and robust (recoveries ranged 61 - 104%, RSD varied from 4.0% to 22.6 %), with a linear analytical range from 0.1 to 10 ug L-1.
This method was successfully applied to natural water samples, collected at five monitoring sites along Suquía River, considering pre and post-application of agricultural pesticides. Most studied pesticides were present throughout the entire period. Nevertheless, post-application period showed concentrations of atrazine, acetochlor, endosulfan, endosulfan sulfate and cypermethrine 1.5 to 5 fold higher than the corresponding to pre-application. As expected, highest levels of pesticides were observed in areas with intensive agricultural practices, being atrazine, cypermetrine and endosulfan sulfate predominant. In urban or recreational areas the prevalent pesticide was cypermethrine. Some values surpasse the National Guidelines for pesticides in freshwaters, pointing out the need of controls in addition to the evaluation of damage to aquatic biota.

EC06P - Sorption and bioavailability in sustainable remediation of organic chemicals

TU 278
Simulating the bioavailability of mineral hydrocarbons for earthworms using different extraction methods
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In the context of assessing the habitat function of contaminated sites only the bioavailable contaminant fraction is of relevance. Information on the habitat function, therefore, can be obtained in ecotoxicological tests performed with representative soil organisms. Depending on the test organism the duration of effect measurements varies between several weeks and several months. In order to obtain results focusing on bioavailability faster, chemical methods are being developed to predict the amount of contaminant that can be taken up by a specific organism. Suitable methods have to simulate the respective exposure pathways and represent the bioavailable fraction of the contaminant. Extraction procedures for mineral hydrocarbons were regarded with respect to their suitability for simulating the bioavailable fraction for soil organisms. As effect parameter the reproduction activity according to OECD Test Guideline 222 was selected. The test was performed in eight sandy soils that had been contaminated with mineral oil for many years. The results obtained in the earthworm studies were compared with the concentration of the mineral hydrocarbon contamination using different extraction procedures: (I) total content using exhaustive extraction (C10 - C40 fraction); (II) fraction of C10-C22 performing exhaustive extraction; (III) water based extraction - shaking procedure (IV) water based extraction - column procedure (V) water based extraction - use of an extra solid phase: HPCD.
The best relation between an increased concentration of contamination and a reduced habitat function was detected for the C10-C22 fraction of the HPCD-extraction. Relations with the C10-C40 fraction of the HPCD-extraction and with the C10-C-22 fraction of the exhaustive extraction were less pronounced. No relation was observed for the water based extractions using shaking and column procedures.
Earthworms are soft bodied organisms and exposed to contaminants mainly via soil pore water and skin. For mineral hydrocarbons the mobile fraction of C10-C22 of the HPCD-extraction seems to be a good indicator for the available fraction. A value of 300 mg/kg may be a suitable threshold value regarding the habitat function of soils. Values exceeding this content may indicate that there is concern for a reduced habitat function.

TU 279
Impact of geosorbents on bioavailability of polycyclic aromatic hydrocarbons to humans by oral uptake
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To assess the health risk resulting from human uptake of contaminated soil from hand-to-mouth activity, it is prerequisite to know the oral bioavailability of the contaminants. Soil-bound contaminants can be mobilized by digestion juices and hence become available for absorption through the intestinal wall [1]. With a physiologically based extraction model (PBET) that is simulating the conditions in the human gastrointestinal tract, it is possible to determine the bioaccessible fraction of soil-bound contaminants.
Previous studies revealed strong variabilities of the bioaccessible fraction (23-68 %) of polycyclic aromatic hydrocarbons (PAH) in natural site samples [2]. Reasons for these variations are unknown. Apart from worldwide use of varying PBET models, we assume that quality and quantity of present geosorbents in the samples lead to variabilities. It is generally known that sorption of PAH in the aquatic environment depends on the type and amount of organic carbon present [3], however, it is unclear if this is also the case in a digestion juice system. The aim of the study is to show differences in bioaccessibility of PAH from single commonly occurring geosorbents. The results are expected to lead to a better understanding of variations in PAH bioaccessibility in natural site samples.
In this study, the geosorbents sand, clay, peat and char coal, which had been characterized in various previous studies, spiked with 16 deuterated EPA-PAH, were extracted with a PBET-model [4]. The bioaccessible fraction of contaminants was analyzed by gas chromatography-mass spectrometry.
The results show that the different geosorbents impact bioaccessibility as expected from the aqueous phase. Pyrene-d10 often showed highest bioaccessibilities: 45.5% ±11.1 (sand), 10.2% ±5.4 (clay), 3.5% ±3.2 (peat) and 0.2% ±0.1 (char coal). As it is known from aqueous sorption-desorption experiments, here also char coal significantly reduced desorption into the human digestion juice. We conclude, that currently bioavailability of PAH from soils and sediments rich in coal, char coal and soot may be significantly overestimated.
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TU 280
Bioavailability measurements in risk assessment - conformity of methods
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Bed sediments often act as a sink for nonpolar organic pollutants released into the aquatic environment. When sources of contamination cease or when emissions are strongly

reduced (e.g. historical pollution), sediments can become a secondary source to their surrounding environment. As a result of the challenges encountered when attempting to predict contaminant bioavailability and mobility, gauging the risk posed by these contaminated sediments remains challenging.
In an effort to control and reduce the impact of contaminated sediment on ecosystems and human health, a risk assessment guideline tool for contaminated marine sediments was recently developed for Norway. In many cases, the comparison of total sediment concentrations with environmental quality standards overestimates the risk posed by these sediments as a result of much stronger partitioning to organic phases in the sediment than commonly assumed. Therefore tools to estimate contaminant bioavailability in sediments, bioaccumulation potential and risk of transfer to the overlying water phase are needed.
This study aimed to assess (predict the potential for) the in vivo bioaccumulation of contaminants from historically polluted sediments using simple generic sorption models and from freely dissolved pore water concentrations measured with passive sampling. Freely dissolved pore water concentrations were measured in batch experiments in the laboratory using low density polyethylene (LDPE). In vivo exposures were carried out using Nereis virens and Hinia reticulata.

TU 281
Bringing (bio)accessibility extractions to the next level - combining mobilisation medium and infinite absorption sink
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Soil bioaccessibility extraction techniques are generally simple dissolution experiments, where the fraction of compounds that is transferred to the medium is measured and considered to be bioaccessible. However, such techniques can lead to an underestimation of bioaccessibility, since they do not account for the consumption of contaminants by either degradation or absorption. It is therefore crucial to develop practical bioaccessibility extraction approaches that combine both mobilisation and consumption processes. The mobilisation medium can be chosen to either maximize desorption without attacking the matrix or even to simulate the relevant organism conditions. A sorbent can act as an infinite diffusion sink for continuously removing the mobilized contaminants from the medium.
Initially, we combined cyclodextrin extraction with a polymer of poly(dimethylsiloxane) and activated carbon which lead to the contaminant trap method[1]. This contaminant trap is a practical and simple approach for the isolation and quantification of the desorption resistant contaminants in soils, its main limitation being that it is not possible to back extract from the polymer. The next step was to find a polymer material and format that can act as infinite sink and allow simple back extraction. Silicone rods were chosen, which are already used in silicone rod extraction and passive sampling. This resulted in a better and more relevant bioaccessibility extraction approach and simplified the analytical procedures. The silicone rods were applied to both cyclodextrin solutions and artificial digestive fluids, using PAHs as model compounds. Passive dosing[2] was applied to determine the free fraction and partitioning of PAHs in cyclodextrin and digestive fluids, which in turn was used for sink dimensioning. Validation experiments with spiked cyclodextrin and digestive fluids confirmed an efficient absorption of PAHs by the silicone rod. Finally, determination of the readily desorbing PAHs from a wood soot sample with and without the absorption sink clearly showed that a sink is needed and makes a substantial difference.

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TU 282
Is there sufficient 'sink' in current bioaccessibility determinations of organic pollutants in soils?
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Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental organic pollutants, a number of which are known carcinogens. Soils are the major environmental sink for PAHs and it has been estimated that over 90 % of the U.K. PAH burden resides in soil. Total pollutant concentration is frequently used in the assessment of risk posed by contaminated land to human health. However, it has been widely established that such an approach may significantly overestimate the amount of pollutant absorbed by humans resulting in an overestimation of risk. One of the main pathways for human exposure to contaminated soils is direct ingestion as a result of hand-to-mouth activity. To address a number several in vitro physiologically-based extraction tests have been proposed for metal and organic pollutants. In recent work we developed a colon extended physiologically-based extraction test (CEPBET), this enhanced the bioaccessibility of PAH compared to a two compartment, i.e. stomach and small intestine, model. The aim of the current work was to combine the CEPBET bioaccessibility test with a charcoal contaminant trap in order to maintain a full diffusion gradient for the desorption process, thereby enhancing the bioaccessibility extraction. This was believed to be necessary because the gastrointestinal tract (GIT) provides a considerable sorptive sink for PAH due to its large surface area and lipophilic nature.
The integration of the contaminant trap significantly enhanced PAH desorption from incubated soils, reducing gut medium concentrations to <5% of the no trap values within the appropriate physiological timescales. This clearly identifies the need for a sink in bioaccessibility extractions. The complete configuration of the CEPBET system is required as the COL significantly increases pollutant desorption. A configuration of CEPBET is now required that combines the 'trap' with the ability to actually measure the bioaccessible fraction. This seems not possible with the present contaminant trap, since quantitative back extraction from the carbon silicone composite is difficult if not impossible. Once an appropriate extractable 'trap' has been found there is the requirement for validation of CEPBET with animal studies.

TU 283
Bioavailability studies: the last available tools for evaluating PAH risks realistically
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Polycyclic aromatic hydrocarbon (PAH) risk assessment is currently overly conservative in the U.S., with Screening Levels that are orders-of-magnitude below anthropogenic background in most urban areas. Additionally, the United States Environmental Protection Agency (USEPA) is proposing to increase the Relative Potency Factors (RPFs) for 5 PAH and to increase the number of carcinogenic PAHs with RPFs from 6 to 26. Several proposed RPFs are 10x or greater including dibenz[a,h]anthracene (10x), benzo[c]fluorene (20x), dibenzo[a,l]pyrene (30x), and benz[j]aceanthrylene (60x). If this approach is adopted, human health risks from exposure to PAHs will increase considerably, and risk-based clean-up levels will drop to below background even for commercial/industrial receptors and a 1x10-4 risk level. It is clear from the literature that the mammalian bioavailability of PAH from weathered soils/sediments is considerably less than 100%. Agencies in the U.S. have increasingly rejected the use of bioavailability adjustments derived from the literature. USEPA has recently supported a policy of performing in vivo bioavailability studies of site-specific media impacted by complex organic compounds. The authors have designed such an in vivo study and are seeking its regulatory approval for execution in 2012. This paper will summarize literature bioavailability results for PAHs and discuss methodological issues regarding the on-going study, which is a robust, internally consistent animal bioavailability study with site aged soils. Issues that will be discussed include type of controls (internal versus external), target PAHs, animal species and strain, sampled biological media (blood, urine, feces, other tissues), pharmacokinetic issues (single time point versus area under the curve), and analytes of interest (parent PAHs versus PAH metabolites vs DNA or protein adducts).

TU 284
Desorption-resistant fraction in PAH-contaminated soils: aged spiked soils can not resemble historically contaminated soils
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Polycyclic aromatic hydrocarbons (PAH) are priority pollutants of soil and groundwater in many countries. Prolonged contact time of PAH and soil constituents may lead to their sequestration, rendering a fraction of contaminants inaccessible for biological processes and diminishing the potential efficacy of bioremediation measures.
The aim of the present study was to evaluate the significance of commonly used spiking protocols for reflecting field conditions in terms of bioaccessibility assessment. Therefore, 25 Austrian soils were collected and spiked with four selected priority polycyclic aromatic hydrocarbons (Phenanthrene, Fluoranthene, Benzo(a)pyrene and Benzo(g,h,i)perylene). PAH desorption behavior from freshly contaminated and aged soils was monitored and compared with PAH desorption from three historically contaminated soils. The non-bioaccessible PAH fraction in the soils was determined using a passive sampling device, the "contaminant trap' recently described by P. Mayer et al [1]. In addition, the soils' infrared spectra and desorption data were incorporated into a multivariate statistical approach (partial least squares regression, PLS) to determine specific soil organic matter regions responsible for PAH sorption.
In the analysis of the non-accessible PAH fraction, the absence of pronounced ageing effects in PAH-spiked, aged soils was noted. After 56 days of desorption time, a distinct desorption resistant fraction occurring in all soils was observed for Benzo(g,h,i)perylene only. Several experimental boundary conditions may serve to explain the lack of an ageing effect, including influences by the contaminant matrix, the extent of sorption site coverage and the contamination's age. Moreover, industrial activity is often adjoined by the enrichment of soils with highly sorbing constituents (e.g. soot, cf. MGP soils). In the present study, weak binding of PAH in spiked soils (aged and freshly contaminated) in connection with the identification of humic acids as possible binding sites via PLS indicate the absence of high-affinity sites (e.g., black carbon) for PAH sorption. This was found for at least 23 of the 25 soils, which were all collected from non-industrialized areas.
The present results indicate that the weight of accessibility data obtained with single-substance spiked soils may have to be re-evaluated.

TU 285
Highways versus pipelines - modelling the contributions of two fungal transport mechanisms to efficient bioremediation
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Based on experimental studies, two fungus-mediated transport mechanisms have been suggested to facilitate the bacterial degradation of organic soil contaminants: bacteria may use liquid films around fungal hyphae for quick dispersal ('fungal highways'), and fungi may take up and translocate contaminants through their mycelial network ('fungal pipelines'). Both mechanisms promise to enhance the bioavailability of contaminants to degrading bacteria. However, a comparative study of their respective

efficiency in increasing biodegradation performance, and its dependence on environmental conditions, has hitherto not been conducted. Using a microbial simulation model, we therefore investigate bacterial degradation performance in response to networks that either act as bacterial dispersal vectors ('highways') or as contaminant translocation vectors ('pipelines') or as a combination of both. We analyse biodegradation improvements compared to the situation without networks, and systematically test a variety of spatially homogeneous and heterogeneous environmental scenarios. Our results suggest that each mechanism can improve biodegradation performance. The degree of improvement, however, may vary distinctly depending on the environmental conditions, and may also be negligible under certain conditions. Particularly, networks acting as 'highways' allow bacteria to overcome motility restrictions and reach remote areas, whereas networks acting as 'pipelines' may initiate degradation by bringing remote contaminants to bacteria. As a consequence, highest biodegradation improvements often emerge from the combination of both mechanisms. We therefore conclude that 'fungal highways' as well as 'fungal pipelines' should be considered for developing novel bioremediation strategies based on fungus-mediated transport. Future experimental studies should focus on detection and appropriate stimulation of the two mechanisms in typical bacteria-fungi associations in contaminated soils.

TU 286

Influence of rhizosphere oomycete mycelia on bacterial biodegradation of phenanthrene present in nonaqueous-phase liquids (NAPLs)

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Two rhizosphere oomycete species (Pythium aphanidermatum and Pythium oligandrum) were tested for their influence on biodegradation of phenanthrene present in NAPLs by the soil bacterium Mycobacterium gilvum VM552. The selected oomycetes showed no antagonistic effects on the bacterium in co-culture assays. An optimized production protocol was established for oomycete mycelia using a direct scrape-out method with solid agar, which prevented the contamination by carbon-rich medium components. Mineralization of 14C-phenanthrene present in hexadecane/fuel-NAPL mixtures by M. gilvum VM552 was increased in the presence of P. oligandrum mycelia. The fungus shortened the lag phase for mineralization and delayed the mineralization plateau. However, there was no stimulation of mineralization when the NAPL was composed of a heptamethylnonane/fuel mixture. We suggest that fungal growth on biodegradable NAPL mixtures may promote the bacterial colonization of the NAPL/water interface, possibly by promoting cell adhesion and/or causing interface fertilization, thus increasing the bioaccessibility of PAHs for bacteria.

TU 287

Sunflowers in rhizoremediation: a possible alternative for improving the bioavailability of PAHs in soils

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The success of the rhizoremediation depends largely on the ability of degraders of contaminants in soil and plant growth that drive organisms to colonize roots efficiently. In our work, we present the effect of sunflower rhizosphere on the biodegradation of PAHs in soil with creosote (21.75 mg-kg^{-1} containing 6 HAPs). We conducted two experiments of biodegradation: one of them in greenhouse with cultivated sunflower and other in the laboratory with suspensions of soil and root exudates of sunflower. The sunflower root exudates were extracted using *in vitro* culture. The presence of the plants caused in greenhouse conditions a selective increase in PAH-degrading populations and a decrease in the concentration of five indicator PAHs. In turn, the root exudates of sunflower had under laboratory conditions a similar stimulatory effect on the degradation of PAHs in soil slurries. The stimulation of the indigenous bacterial population resulted in both situations in a similar residual concentration of PAHs. We can conclude that the effects of plants on the residual concentrations of PAHs could be reproduced under laboratory conditions by slurring, shaking and the addition of root exudates.

TU 288

Combining synchronous fluorescence, liquid scintillation and passive dosing for studying the bioavailability of pyrene in the presence of dissolved humic substances

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Degrading bacteria usually take up dissolved polycyclic aromatic hydrocarbons such as pyrene, by diffusion from the surrounding aqueous phase. Here, the dissolved concentrations determine bioavailability, and low freely dissolved concentrations, sometimes, can cause slow biodegradation rates. When dissolved humic substances (HS) are present, it is important to know if the association with HS affects the concentration of freely dissolved pyrene and how this affects biodegradation rates. We have applied a new combination of analytical techniques (synchronous fluorescence spectrometry, scintillation counting) to establish the dynamics of ^{14}C-pyrene in the aqueous phase during biodegradation, in the presence and absence of HS isolated from soil. We also used a technique based on the principle of passive dosing to control the dissolved concentrations of pyrene by partitioning from a preloaded polymer (polydimethylsiloxane). The results indicate that the establishment of an appropriate chemical activity gradient is critical for a positive or negative influence of HS on biodegradation.

TU 289

Biosurfactants and sustainable bioremediation: effects on slow desorption PAHs

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Slow desorption from soil and sediments is a critical limiting factor that controls biodegradation rates of hydrophobic pollutants such as PAHs, resulting in a long-term persistence of these compounds in the environment. The use of biosurfactants is a promising alternative for enhancing desorption of soil-sorbed PAHs and their bioavailability for microbial degradation. It is already known that biosurfactants can promote dissolution of solid PAHs and also enhance desorption when they are present as rapidly desorbing fractions (> 0.1 h^{-1}). However, not much is known about the effect of biosurfactants on bioavailability of slowly-desorbing hydrophobic compounds, as PAHs. In this study, we focus on the capacity of rhamnolipid biosurfactants, produced by *Pseudomonas aeruginosa* 19SJ, to enhance the bioavailability of different soil-sorbed ^{14}C-labeled PAHs in soil-aqueous systems. In a well-controlled batch system, we studied the effect of biosurfactants, at concentrations above the critical micellar concentration (CMC), on the biodegradation of fast and slowly-desorbing fractions of ^{14}C-labeled PAHs present in soil. Desorption kinetics of ^{14}C-PAHs from soil were performed by Tenax solid-phase extraction and compared to mineralization kinetics by a PAH-degrading soil bacterium (*Mycobacterium gilvum* VM552) in the presence of biosurfactants. Results indicated that the promoting effects of biosurfactants on biodegradation were accentuated in soils exhibiting a slow-desorption profile. Given the biodegradable and non-toxic nature of biosurfactants, their use constitutes a promising alternative for promoting bioavailability of this 'resistant' fraction in a sustainable way.

TU 290

Bacterial taxis and sustainable remediation: effects on bacterial dispersal

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We focus in modulating the deposition and motility of degraders in porous media as a bioavailability-promoting strategy. The positive effects of - often energy-consuming - mobilization approaches in bioremediation depend on the efficiency of bacterial movement in porous media, which is often restricted by high deposition rates and adhesion to soil surfaces. Besides, adhesion to surfaces may be sometimes beneficial in promoting biodegradation of slowly-desorbing chemicals. In well-controlled column systems, we assessed the influence of different chemoeffectors on deposition of a chemotactic, naphthalene-degrading bacterium (*Pseudomonas putida* G7). We also characterized the motility behavior by capillary assays and by analyzing the movement of individual cells through computer-assisted motion analysis, determining parameters like linear speed and rate of change of direction. The cell interaction with the column packing material, and subsequently deposition, depended on the motile behaviour (hypermotility, attraction or repellence). For example, positive chemotaxis caused smooth trajectories and reduced the affinity of the cells for surfaces, thus promoting transport due to a decreased deposition, whereas repellence caused the opposite effect. We propose that, by promoting the right motility behaviour, we can adapt cell deposition to a given bioremediation scenario, thus achieving an enhanced and sustainable bioremediation performance.

TU 291

Behavioural responses of Tetrahymena pyriformis exposed to microgradients of hydrophobic organic chemicals

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Behavioural changes of microorganisms may reveal sublethal adverse effects of hydrophobic organic chemicals. Using them as an endpoint requires, however, that the organisms can be observed microscopically during exposure. We therefore applied passive dosing on microscope slides as a new experimental platform to study the effect of selected polyaromatic hydrocarbons (PAHs) on the behaviour of the ciliate Tetrahymena pyriformis. Motile cells and organisms that are able to sense chemical signals may direct their movement along concentration gradients of a chemical, a behaviour known as chemotaxis. T. pyriformis was therefore exposed to various PAH-gradients and the swimming behaviour of the cells was then recorded under infra-red light illumination. Trajectories of the cells were obtained using an automated cell tracking software and were subsequently analyzed for chemotaxis by statistical measures. The Taylor model, which mathematically describes the scale-dependent transition from ballistic to diffusive movements of particles in suspension, was applied here for the first time in an ecotoxicological context to obtain motility parameters such as the tumbling frequency and effective swimming velocity to evaluate changes in the swimming behaviour of the cells. The results did not reveal chemotactic behaviour of T. pyriformis in the concentrations gradients of the compounds tested. However, motility behaviour was affected at levels that were about two orders of magnitude below the reported effective chemical activity causing 50% lethality, emphasizing that behavioural responses are a sensitive endpoint in toxicity testing.

TU 292

Sorption of ionized and polar organic contaminants to natural dissolved organic matter

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Concentrations of dissolved organic matter (DOM) have been rising in the environment over the past decades due to climate change and decreases in acid rain. The transport potential of organic contaminants can be affected by increased DOM concentrations in surface water. It is therefore relevant to gain more insight into the mechanism of organic contaminant sorption to DOM. In this study, the interactions between polar organic contaminants (neutral and ionized forms) and DOM were studied to assess the

influence of charge and molecular structure on the sorption process. Representative emerging contaminants were selected, covering hormones, pharmaceuticals, personal care products, and pesticides. Batch sorption studies were conducted with natural DOM-containing water samples obtained from Finland, United Kingdom, and the Netherlands and by using polyacrylate passive samplers to determine aqueous contaminant concentrations. The DOM samples were characterized by chemical and physical fractionation methods. The results indicated that charge and molecular size of ionized compounds dominate sorption to both DOM and polyacrylate. Positively charged compounds showed a strong pH dependent sorption to DOM, whereas the neutral forms were the most important species responsible for sorption to polyacrylate. Negatively charged compounds showed the lowest affinity to both DOM and polyacrylate. For most neutral compounds investigated, the water phase represented a more favorable medium compared to DOM. The obtained results may increase our knowledge on the sorption of polar organic contaminants to DOM, which is currently based on only limited data.

TU 293

Binding can increase the mobility and uptake of hydrophobic organic compounds

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The binding of hydrophobic organic compounds (HOCs) is usually considered to be a risk reduction process. However, if the bound form becomes mobile then it can in fact enhance uptake and risk. This phenomenon can been seen in 1) colloid facilitated transport 2) the use of chemical agents to enhance soil remediation and 3) in biology, where body fluids can enhance the release of HOCs from soil and consequently increase their bioaccessibility.
In the current work we studied the above examples using "enhanced capacity"as a new measurement endpoint. Enhanced capacity (E) has some similarities to the well-established concept of "solubility enhancement", but should not be confused with it. Solubility enhancement is determined at, and applies only to, the saturation level of the HOCs, whereas E can be determined at or below the saturation, and for defined levels of each mixture component. This means it refers to a more relevant range of environmental concentrations and mixture compositions. Passive dosing method[1] was applied to determine E for HOCs of 1) soil leachates collected from tile-drains of sandy clay loam and sandy loam fields of the Danish Pesticide Leaching Assessment Programme 2) sodium dodecyl sulfate and hydroxylpropyl-β-cyclodextrin that are used as chemicals in soil remediation technology and 3) artificial digestive fluids used in in-vitro test for assesing bioaccessibility of soil-bound PAHs[2]. The use of passive dosing circumvented artefacts associated with adding crystals of HOCs into an aqueous solution, and due to the low relative standard deviation of the method even small enhancements could be measured with high precision.
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TU 294

Dynamic passive dosing for studying microbial PAH degradation: a comparison of experimental and model results

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Biodegradation plays a key role in PAH fate, and understanding kinetics as a function of (bio)availability is critical for elucidating their persistence. However, biodegradation mainly occurs in an aqueous environment, posing technical challenges for producing reliable kinetic data because of low PAH solubilities and sorptive losses. To overcome these, an experimental approach based on dynamic passive dosing is presented which: avoids using co-solvent for introducing the PAHs, buffers substrate depletion so biotransformation is measured within a narrow and defined concentration range and enables high compound turnover to simplify biodegradation measurements even at realistically low concentrations. The biodegradation kinetics of two PAHs by the bacterium *Sphingomonas paucimobilis* EPA505 were measured at defined dissolved concentrations ranging over 4 orders of magnitude, from 0.115 to 865 μg L^{-1} for phenanthrene and 0.028 to 141 μg L^{-1} for fluoranthene. Both compounds had similar biodegradation rates, and these increased in line with higher dissolved concentrations. First-order biodegradation rate constants were also similar for both, but these decreased at higher dissolved concentrations. The experimental results were compared to those obtained using different modelling approaches, including simulations based on the Best equation. These show a good agreement at the lower dissolved concentrations, whereas at higher concentrations the experimental measurements were lower than predicted, and indicate that that other factors such as PAH toxicity or essential nutrient availability play a role. Therefore, combining measurements of PAH biodegradation kinetics at defined concentrations using dynamic passive dosing, and their interpretation by modelling is a useful tool to further understand their bioavailability, biodegradation and persistence.

TU 295

Enhanced mass transfer of hydrophobic organic compounds from NAPLs into the aqueous phase by dissolved organic carbon

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Hydrophobic organic compounds (HOCs) are often found as mixtures in the form of non-aqueous phase liquids (NAPLs). Due to their hydrophobic nature, the HOCs preferentially remain in the NAPL, with slow mass fluxes into the aqueous phase. However, since water dissolved HOCs play a key role in diffusive uptake into organisms, microorganisms using HOCs as a source of carbon and energy face a large reservoir of inaccessible food in the NAPL which is often reflected in slow bioremediation of NAPL contaminated sites. Interestingly, mobile "colloidal-like"phases can contribute to diffusive mass exchange processes between surfaces and the bulk aqueous phase, a phenomenon termed enhanced or facilitated diffusion. Therefore, this study investigated the role of dissolved organic carbon (DOC) in enhancing the mass transfer of hydrophobic organic compounds from NAPLs into the aqueous phase above that attributable to dissolved molecular diffusion alone. In controlled experiments, mass transfer rates of five NAPL-phase PAHs (log KOW 4.15 - 5.39) into the aqueous phase containing different concentrations of DOC were measured. Mass transfer rates were increased by up to a factor of four in the presence of DOC, with the greatest enhancement being observed for more hydrophobic compounds and highest DOC concentrations. These increases could not be explained by dissolved molecular diffusion alone, and point to a parallel DOC-mediated diffusive pathway. The nature of the DOC-mediated diffusion pathway as a function of the DOC concentration and PAH sorption behaviour to the DOC was investigated using diffusion-based models, and found to increase with DOC concentration and compound sorption. Therefore, for "super"hydrophobic compounds this pathway could both dominate but also increase mass transfer rates by orders of magnitude, even at environmental DOC concentrations. This has important implications for their bioremediation, as well bioconcentration and toxicity.

TU 296

On the effect of vitamins and nutrients on the solubilization of petrodiesel/biodiesel blends in water

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The effect of mineral salts on the aqueous solubility of nonelectrolytes is largely assumed to exhibt the "salting out"or "salting in"effects in which the aqueous solubility of nonelectrolytes is decreased or increased upon the addition of salts. Pioneering investigations by Setschenow [1] and later by Carter and Hardy [2] among others, resulted in semi-empirical relationships that describe the dependence of the solubility of nonelectrolyte solutes on salts concentrations. We conducted equilibiation experiments of petrodiesel/biodiesel blends (B0, B20, B40, B60, B80, and B100, where B100 is 100% biodiesel) in water in the presence and absence of standard vitamins and nutrients used for bacterial growth studies. The solubility of aromatic compounds was found to be in agreement with the salting out effect in the presence of the added nutients. The solubility of the C10 - C21 n-alkanes, however, exhibited very odd and complex behavior and was found to be significantly enhanced ($p < 0.0005$), up to 40-fold in the presence of the fatty acid methyl esters (FAMEs) and the vitamins and nutrients meduim, compared to FAMEs and deionized water alone. A similar observation was made by Baker [3] regarding the effect of salts on solublization of the alkanes and aromatic compounds in the presence of fatty acid soap solution. They reported that, in the presence of sodium chloride in dilute fatty acid soap solutions, the solubility of paraffin hydrocarbons is markedly higher and that of aromatic hydrocarbons is lower. Those observations are found to contribute significantly to the bioavailability of the n-alkanes in the water column. Biodegradation studies conducted in our laboratory showed significant enhancement for the microbial utilization rates of the n-alkanes in the petrodiesel/biodiesel blends, which we interpret to be due to the increase in aqueous concentrations of the n-alkanes in the presence of the FAMEs and nutirents.

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TU 297

Photo-transformation of 2,3,7,8-TCDD in presence of natural organic matter studied by in vitro bioassay

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a representative of hydrophobic organic compounds (HOCs, frequent anthropogenic environmental pollutants comprising also various pesticides), can cause many adverse effects in organisms. Activation of arylhydrocarbon receptor (AhR) can be very important mechanism of toxicity for so called "dioxin-like"HOCs. Natural organic matter (NOM), being naturally occurring compounds (up to 50 mg/L in waters), occurs together with HOCs in contaminated water. Beside other important ecological properties, NOM serves as a natural source of reactive oxygen species that are formed after NOM irradiation. Direct photolysis of HOCs is a very important way of their degradation in the aquatic environment. Nevertheless, oxygen species, formed after HS irradiation, can theoretically enhance the photochemical degradation of HOCs. In present study, we have assessed the ability of various NOM concentrations to enhance photo-degradation of TCDD. Aqueous TCDD+NOM

solutions were irradiated by sun light in quartz tubes for up to 3 days. Photo-degradation of TCDD studied by in vitro assay (based on the H4IIE-luc transgenic cell line) was observed, but both TCDD alone and TCDD in mixtures with low concentrations of NOM (up to 10 mg/L) were photo-degraded in almost the same half-lives. High NOM concentrations (25-150 mg/L) have significantly extended the half-lives of TCDD photo-degradation, probably due to filter effect of more coloured solutions. Supported by projec CETOCOEN (European Regional Development Fund no. CZ.1.05/2.1.00/01.0001).

TU 298

Influence of sorption on bioavailability and biodegradation of secondary alkane sulfonates (SAS) in marine sediments

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Coastal marine ecosystems are often influenced by wastewater discharges from surrounding populations. Surfactants, with a worldwide production over 10 million tons per year, are among the organic contaminants showing highest concentrations in wastewater. Most available studies on this topic deal with the distribution and fate of alkylphenol polyethoxylates (APEO) in aquatic systems, as some of their degradation intermediates are endocrine disruptor compounds (EDC). Linear alkylbenzene sulfonates (LAS) have also been extensively studied as they show the highest worldwide production volumes. Environmental data on many other surfactants, however, are still scarce. This is the case of secondary alkane sulfonates (SAS), one of the major anionic surfactants used in the market of dishwashing, laundry and cleaning products. Although this compound is easily removed during wastewater treatment, previous studies have shown that SAS and other anionic surfactants such as LAS accumulate in sludge as they are hardly biodegraded during anaerobic digestion. Recently, anaerobic degradation of LAS was confirmed in marine sediments due to the presence of sulfate reducing bacteria. This research has focused on determining whether SAS are biodegradable or not in absence of oxygen in the marine environment, and, if that happens, on the role of sorption on the speed of the biodegradation. First, sorption experiments were performed using several amounts of sediments (0.5-5 g) and SAS concentrations (1-10 mg/kg). Distribution coefficients ranged from 54 to 1505 L/Kg depending on the SAS homologue considered. Thus, we observed that the sorption capacity was much higher for those homologues having longer alkyl chains (e.g., C17-SAS) rather than for more polar homologues such as C14-SAS. Later, biodegradation experiments using anoxic marine sediments and seawater were conducted. SAS anaerobic biodegradation was observed for the first time, reaching overall values up to 98% in 166 days. Half-life values ranged from 20 days (C14-SAS) to 37 days (C17-SAS), showing that the speed of this process significantly depends on the sorption capacity and, therefore, bioavailability, of each SAS homologue.

TU 299

Remediation of PCB- and PAH- contaminated soil with modified clays

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Contamination of soils and sediments by polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PCBs) is a major concern worldwide. This is connected with their persistence and the threat they pose to the environment and human health. Development of efficient and reliable strategies to reduce PCB and PAH concentrations in contaminated soils has been a crucial task for scientists. Immobilization of these organic contaminants seems to be preferred since total removal of pollutants is hardly possible. This study is therefore aimed at evaluating the potential of modified clay minerals (with surfactants, humic acid and papaya seed) in the retention of PAHs and PCBs in soils.

Sodium dodecyl sulphate (SDS) and humic acid were used to modify bentonite. Kaolinite was modified by Papaya Seed. Different percentages of the modified clays were mixed with the contaminated soil and suspended in 0.01M CaCl2 in the dark for twenty days. After equilibration analysis of PCBs and PAHs in solution was carried out using SPME coupled with GC/MS.

Presented are the sorption capacities of the modified clays and results of remediation. The results of this study indicate a promising potential of the modified clays to immobilize PCBs and PAHs in soils.

TU 300

Fate of iodinated X-ray contrast media in a soil column percolation experiment simulating elevated dissolved organic carbon (DOC) by amendment of saccharose

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Iodinated X-ray contrast media (X-RCM) occur in concentration ranges of several hundred ng/L in many surface waters used for drinking water production. To clean up surface water, sustainable water treatment techniques like river bank filtration or artificial groundwater recharge (AGR) are used, but some X-RCM like amidotrizoic acid are known to be very persistent. Due to low the sorption tendency of X-RCM [1], their fate is rather related to biodegradation, as proved by the detection of metabolites reported in literature [2].

To simulate the process of AGR, the fate of six iodinated X-ray contrast media ioxithalamic acid, iohexol, iomeprol, iopamidol, amidotrizoic acid and iopromide was studied during percolation of a sandy gravelly substrate in a column percolation experiment using filtrated surface water from the Rhine. The question of interest was, whether elevated DOC would improve or impair the removal of X-RCM during the column passage. Therefore, filtrated Rhine water used for column percolation was spiked with concentrated aqueous saccharose solution to achieve elevated DOC and to simulate the pollution of the Rhine with low molecular organic compounds, e.g. from food industry or accidental spills. Time weighted composite samples of column influent and effluent were analysed for X-RCM and DOC and treatments with and without saccharose amendment were compared.

Moderately elevated DOC (1.3-fold of background) stimulated iohexol removal, but for most X-RCM there was no effect. In contrast, twenty-fold elevated background DOC impaired the removal of most X-RCM, while there was a tendency towards elevated iopamidol removal. The latter stimulation is supposed to be related to the observed changes in redox settings in the column induced by the high DOC load, as the fate of iopamidol is reported to depend on site-specific redox conditions at several field sites in literature [1].

Regarding the removal of X-RCM, moderate pollution of surface waters with low molecular organic compounds can thus be compensated by natural water treatment like AGR, but excess pollution will reduce the cleaning capacity of these systems and must be avoided.

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TU 301

Effect of pH and electrolyte concentration on soil adsorption of pesticides

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The Japanese Positive List System for Agricultural Chemicals was implemented in 2006. Under this system, the uniform limit was set at 0.01 mg/kg for agricultural chemicals for which maximum residue limits (MRLs) have not been established. Since then, some agricultural chemicals have been detected in some vegetable crops at levels above the uniform limit, and the distribution of these crops has been prohibited. One of the reasons why the limit has been exceeded is that succeeding crops are contaminated by pesticides remaining in the soil. Assuming that these pesticides in the soil are taken up by plants via the soil solution, an understanding of the soil-water distribution of the pesticides is important to predict crop pesticide residues. Soil adsorption of nonionic pesticides is strongly influenced by soil organic matter content. However, there is little information available on the influence of soil pH and electrolyte concentrations, changed by chemical fertilizer treatment, on pesticide adsorption. We therefore used batch experiments to investigate the effects of these factors on the distribution coefficient for adsorption (Kd) of about 30 pesticides. We used buffer solutions (pH 3, 5, 7.5, and 9) and CaCl2 solutions (0.01, 0.1, and 1 mol/L) and five soils with different organic matter contents. Each soil was shaken with an aqueous solution containing 0.1 mg/L of pesticide at a soil-to-solution ratio of 1 to 5, with a 24-h equilibration period for all experiments. Increasing the CaCl2 concentration from 0.01 to 0.1 mol/L had no significant effect on soil adsorption of pesticides, but the Kd values with 1 mol/L CaCl2 solution were slightly higher than those at the other two concentrations. Pesticide adsorption was greatly affected by pH level. In general, Kd values were negatively correlated with pH levels, and the rate of Kd change, calculated by dividing the Kd values at pH 3 by those at pH 9, was greater in the case of soils with higher organic carbon contents and of pesticides with higher octanol/water partition coefficient (log Kow). The results suggest that the hydrophobic interactions between pesticides and soil organic matter are influenced by pH levels. Further, it is implicated that crops can take up the pesticide from soil at high pH compared with at low pH.

TU 302

Relationships between soil adsorption of pesticides and pesticide/soil properties

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The positive list system for pesticide residue in food, such as crops, was applied in Japan in 2006. For pesticides for which maximum residue levels were not set, the residue levels in food must not exceed 0.01 mg/kg (uniform limit). Pesticides, which are applied to foliage and soil at the crop cultivation, remain in soil after harvest, and pesticides remaining in soil may contaminate the succeeding crop. Crops, for which growing cycle is short, are rotated in Japan. Therefore, the residue levels of some pesticides in succeeding crops have exceeded 0.01 mg/kg in Japan. To prevent excess of the residue level 0.01 mg/kg in the succeeding crop, the succeeding crop must be cultivated in consideration of1) the physicochemical properties of the pesticides applied to the preceding crop, 2) the soil properties affecting the pesticide residue in soil, and 3) the pesticide's uptake into the succeeding crop.

The succeeding crop may take up the pesticide in soil solution from the root or the stem as a kind of a pesticide's uptake into the crop. Data on the distribution of a pesticide in soil/solution (pesticide soil adsorption) is important for assessing the risk of pesticide contamination of the succeeding crop. Soil adsorption of nonionic pesticides

is influenced by organic carbon content in soil; however, there are few investigations on influence of pesticide and soil properties except organic carbon content on the adsorption of pesticide in soil.
The present study conducted batch experiments with approximately 30 pesticides and 7 soils in Japan to determine their soil adsorption coefficients (Kd values). The results of relationships between Kd values and pesticide/soil properties will be present.

TU 303
Leaching of aged DDTs and current use pesticide residues in undisturbed soil columns: non-ionic surfactant and carboxylic acids effects
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Soil pollution by Persistent Organic Pollutants such as DDTs (*p´p´*-DDT and its metabolites *p´p´*-DDE and *p´p´*-DDD) represents a continuous source to the environment. As a consequence of their high persistence DDTs occur up to 60 cm on agricultural soils. The Current Use Pesticide (CUP) endosulfan is characterized by its high lixiviation potential due to its relatively low K*ow* value. Introduction of riverine water on polluted soils during irrigation or flooding events as well as plant growing may modify pesticide availability and leaching. Surface waters receive discharges from industrial, urban and agricultural uses that will account for a variety of compounds such as surfactants, colloids and dissolved organic carbon. This work studies the vertical distribution and movement of aged DDTs and endosulfan in undisturbed soil columns irrigated with: 1-control (riverine water), 2-Tw-80 (riverine water + Tween 80 0.5 cmc), and 3-acids (riverine water + sodium citrate and sodium oxalate, 0.05 M). Experiments were carried out with 13 cm x 30 cm soil columns taken from Aridisol soils from a fruit field in Patagonian, Argentina. Columns were saturated with distilled water and solutions (4L) were added under saturated condition. One L leachates (n=4) were collected for pesticide residues analysis. After elution, columns were dried (24 h), opened and sampling on 5 cm sections. Pesticide desorption was performed by batch technique and residues analysis on soil and water by GC-ECD. *p´p´*-DDE>*p´p´*-DDT>α-endosulfan>β-endosulfan pattern was found in all soil sections. *p´p´*-DDE levels ranged between 7-250, 8-70, and 13-200 ng g^{-1} dry weight in control, Tw-80 and Acids, respectively. Under all conditions pesticide levels decreased concomitantly with depth and sand increase. In control and Acids, *p´p´*-DDE and *p´p´*-DDT desorption from soil was correlated with their levels in soil ($r>0.8$ $p<0.02$). Total pesticide levels in elution waters followed the order Tw-80>Acids>Control. Under control or Acids, α-endosulfan was the main compound on the first elution volume and *p´p´*-DDE started to lixiviate after 2 L while for Tw-80, DDE represents the main residue in all elution waters. Lixiviation of aged *p´p´*-DDT residues or endosulfan effectively occurs under irrigation with riverine water and is increased by adding non-ionic surfactant or carboxylic acids. Tw-80 had a washing effect of pesticides leading to lower levels on soil while carboxylic acids enhance pesticides availability.

TU 304
Sorption of cationic organic compounds to soil as the sum of soil components
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Many emerging contaminants are strong bases that under environmental conditions mostly occur as cationic species. The environmental fate of these compounds depends on sorption from water to particulate matter, such as soil, sediment and dissolved colloids. Since both natural organic matter and clay minerals are negatively charged substrates, both sorbent types are well known to bind organic cations, but it is not clear to what extent each type contributes in natural soils. Using a customized dynamic HPLC column retention method, we studied the sorption of a set of organic cations to individual soil components, such as natural organic matter (NOM, Pahokee peat) and pure clay minerals (kaolinite, illite, bentonite). In addition, sorption affinity to five different Eurosoils was tested, which ranged in organic carbon content (fOC) from 1.3 - 9.2%, and in clay content from 6 - 75%. We examined to what extent sorption to natural soil can be predicted based on sorption to individual soil components alone. In general, sorption of primary amines to clay minerals was weak relative to quaternary ammonium compounds (quats), whereas sorption of primary amines to NOM was strong relative to quats. Such relative sorption affinity differences between test compounds between NOM and clay minerals were used to evaluate soil sorption.

TU 305
Degradation of UV filters in sewage sludge by the ligninolytic fungus Trametes versicolor and study of the 4MBC degradation process in liquid medium
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Sunscreens agents, also known as UV filters, have become very popular chemicals since they were shown to have a protective role against photoaging, photocarcinogenesis and photoimmunosupression promoted by UV sun radiation. These compounds are extensively used in personal care products but are also present in a wide variety of industrial goods such as textiles, paints, or plastics to prevent photodegradation of polymers and pigments. UV filters can enter the environment through the liquid effluent of wastewater treatment plants (WWTPs) but also absorbed in the sludge due to their high hydrophobic character, and later spread on agricultural fields. A solid-state treatment of WWTP sludge with the white-rot fungi Trametes versicolor is reported in the present work as feasible to degrade them in a range from 87 to 100%. This experiment is the first step of any future fungal treatment, essential to prove that the elimination is only due to the action of the fungus and not of other microorganisms as the sludge was previously sterilized.
Degradation studies of 3-(4'-methylbenzylidene) camphor (4MBC) in liquid media were also done in particular and complete removal was achieved in less than 24 h. The main metabolites were identified and the first steps of the transformation pathway were elucidated: a mono- or di- hydroxylation by cytochrome P450 and the subsequent conjugation with a pentose. These results support the extended hypothesis that conjugation processes constitute one of the defensive mechanisms that fungi activate against toxic hydroxylated compounds.

TU 306
Concentration levels of phtalates in water and sadiment samples from Nadela river basin, Vojvodina region
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Phtalates belong to the group of dominant industrial pollutants and are ubiquitous overspread class of compounds with low water solubility/high fat solubility and low volatility. Due to their low water solubility, the phthalates hydrolyse relatively slowly, but the actual dynamic rate varies according to temperature, concentration and solubility. The physical and chemical properties of the phthalates have made them suitable as plasticisers in polymers such as plastic and rubber. Among the phthalates diethylhexyl phthalate (DEHP) predominates and has many possible applications, especially for PVC. The highest concentrations of DEHP are found in products for flooring, foil and plastic-coated fabrics. Phthalates are also included as plasticisers for binders in different kinds of paint and adhesives. Within the Project the concentration levels of phthalates in water and sediment from Nadela River were determined. Nadela basin is situated near city of Pancevo, Vojvodina region, and is covered by numerous industrial sites and can represent the significant source of these pollutants. The field survey was done in 9 sampling sites in the catchment area of the Nadela in the early spring 2009. The laboratory analysis were conducted in Environmental Institute, Kos, Slovak Republic and in the laboratories of Institute for Public Health of Vojvodina. All water samples were positive on presence of four of the six selected phthalates: di-n-ethyl phthalate (DEP), diisobutyl phthalate (DIPB), di-n-butyl phthalate (DBP), and DEHP. The concentrations varied in the range: for DEP from 0.01µg/l till 0.2 µg/l, DIPB from 0.08 µg/l till 0.95 µg/l, DBP from 0.3 µg/l till 2.5 µg/l and DEHP from 0.7 µg/l till 2.987 µg/l. In sediment, three of selected phthalates were determined in all sampling sites. All tested samples were positive on DIBP in the range of 24 - 80 µg/kg, DBP in the range of 215 - 996 µg/kg and DEHP in the range of 124 - 779 µg/kg. On 5 of 9 sampling sites the concentration levels of DEHP exceeded EQS and this phthalate is listed to be a human carcinogen. In the case of sediments concentrations of standards have not been exceeded in any sample. Based on the analytical results and compared with the concentration levels obtained for other priority substances, it can be concluded, that DEHP is the most problematic priority substance in the Nadela hydro-system basin.

The work was supported by Ministry of Education and Science, Republic of Serbia (III46009 and TR34014).

TU 307
Increasing levels of PBDEs, PCBs and organochlorine pesticides in fish following a mega-flooding episode in the Negro River basin, Argentinean Patagonia
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A mega-flooding event in the Negro River basin, Argentinean Patagonia, caused a significant outflow of contaminants from the surrounding soils. Agriculture, hydroelectric centrals and industrial-urban conglomerates are the most important activities in the basin. This study evaluates the effects of flooding event on polybrominated diphenyl ethers (PBDEs), endosulfans, DDTs and polychlorinated biphenyls (PCBs) levels in females of silverside (Odontesthes hatcheri) tissues. Muscle, liver, gills, gonads and stomach content were analyzed by GC-MS. Levels in water, suspended particulate matter (SMP) and sediments (SS) were also measured. Post- and pre-flood fish showed the following pattern: DDTs>PCBs>endosulfans≥PBDEs. Post-flood fish showed the highest contaminants levels (ng/g lipid), at expenses of PBDEs and PCBs. PBDEs showed the greatest difference between post- and pre-flood fish (up more than 240 times). BDE-47 was the predominant congener in all tissues; however BDE-100 and BDE-99 showed the highest increase in post-flood liver (up to 400 times). The dominance of BDEs 47, 99 and 100 was consistent with the general pattern found in abiotic samples and fish of the same area, as well as denote the use of technical mixtures of penta-PBDEs. PCBs levels in post-flood fish increase until 23 times (from 63.2 to 1,478.6 ng/g lipid in liver), which were dominated by penta- (PCB-110, 118) and hexa- (PCB-153, 138) congeners. These results could have stemmed from historical usage of Arochlor 1254 and 1260 in Argentina. In post- and pre-flood fish, endosulfan sulfate presented the highest levels suggesting metabolic activity in addition to direct metabolite uptake, while an a-/b- ratio >1 similar to the technical mixture which is widely used in the region. In post-flood muscle, DDTs levels increased 5-fold than pre-flood. The metabolite p,p'-DDE represented about 80% of DDTs. This pattern would be a result of the intensive use of this insecticide during long time on agricultural practices. Contaminant profiles observed in pre- and post-flood silverside were according to water, SPM and SS, showing that this specie is a good biomonitor of aquatic pollution of Negro River.The presence of pollutants in this river was modified and enhanced by the flooding, increasing their bioavailability to fish. Additionally, pollutant occurrence deserves more attention, and monitoring programs are recommended in order to diminish their incorporation to aquatic ecosystem.

TU 308
Polychloro-dioxins, furans and biphenyls in fish, crabs and clams from the San Jacinto River Waste Pits, TX
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Fish, crabs, and clams were collected from the San Jacinto River waste pits, a superfund site in Houston, TX and analyzed for polychlorodibenzo-p-dioxins, polychlorodibenzofurans (PCDD/Fs) and dioxin like polychlorobiphenyls (dl-PCBs). Sample preparations comprised of tissue homogenization, enhanced pressurized liquid extraction and cleanup, and concentration. Isotopically labeled surrogates were spiked prior to extraction and were used to correct for target analytes loss during sample preparation. Samples were analyzed employing gas chromatography negative chemical ionization mass spectrometry. The method detection limits ranged from 2.0 to 10 pg/g ww in fish tissues. Ten out of twelve high priority dl-PCBs (Identified by World Health Organization, 2005) were measured with concentrations ranged from 2.0 to 948 pg/g ww. Average dl-PCBs concentrations were at least an order of magnitude higher than that of PCDD/Fs. Average PCDD/Fs fish tissue concentrations were at least an order of magnitude than that of crabs and clams. TEQclam was found to be at least 25X higher than TEQfish. Biomagnifications factor and Biota Sedimentation Accumulation Factors were also calculated.

TU 309
Effect of humic substances on remediation of soil- phosphogypsum mixtures
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Phosphogypsum (PG) is waste material in industrial production of phosphoric acid. Due to high content of nutrients (calcium, phosphorus and sulfur) it is sometimes used as a fertilizer for poor unfertile soils. But besides nutrients, PG also contains toxic impurities, such as fluorine, strontium and some others. One of contemporary tools to reduce the ecotoxicity of polluted soils and soil-PG mixtures is an application of humic substances (HS). The objective of this study was to evaluate the detoxifying ability of a number of humates towards PG applied to model soil mixtures.
Model soil (MS) contained (wt, %) 20% of kaoline, 10% of peat and 70% of sand with particle size 0,2-0,4 mm (ISO 11268-1). PG was carefully mixed with MS at concentration 3.3 and 7.5 wt, %. Humates from peat - Pe-FlexK, Pe-EcoK, coal - BC-EnK, BC-HumNa and lignosulphonate - OW-LhK introduced into the MS in the form of an aqueous suspension (distilled water) to achieve a concentration in soils 0.005 and 0.020 wt, %. The ecotoxicity was evaluated in water extracts using three species of test-organisms from different taxonomic groups: microalgae *Scenedesmus quadricauda* (decrease of cells population growth, 72h), crustaceans *Daphnia magna* (mortality, 96h), and higher plants *Sinapis alba* (decrease of root elongation, 120h). Toxicometric parameters (EC_{50}- the median PG concentration that caused a 50% test-reaction reduction, and NOEL - no observed effect level - the PG concentration that caused test-reaction reduction below the toxic level) and detoxification indexes *D* were calculated.
Analyses of average detoxification indexes *DAV* (averaged over three test-organisms) showed that influence of humates depended both on PG concentration and humates' nature. Thus, application of PG at concentration 7.5 wt, % was toxic for all the test-cultures at all treatments and humates were not able to decrease the toxicity. At lower rate of PG (3.3 wt, %) some humates decreased the toxicity (*DAV* is 0.5-0.8), whether other did not. Among all humates tested the highest detoxifying ability was observed for two samples: Pe-FlexK from peat and BC-EnK from coal. For these humates EC_{50} and NOEL values increased in 1.3-1.8 times for *S. Quadricauda*, in 1.1-1.4 times for *D. Magna*, in 1.6-2 times for *S. alba* for 0.005 wt, % humates content.
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ET01P - A Systems Biology approach to predictive Ecotoxicology

TU 312
Birds species versus crops: a GIS-based procedure to identify specific combinations
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Regulation (EC) No 1107/2009, applied from 14 June 2011, requires new data protection rules for both active substance and product data and, for the first time, includes data protection for extension of authorisations to minor uses. Moreover, rules for either avoiding duplication or sharing tests and studies involving vertebrate data have been included. Article 62, in particular, introduces new vertebrate data sharing provisions which allows Member States to use vertebrate studies on behalf of prospective applicants if an agreement with the holder(s) of the authorisation cannot be reached.
Considering birds, the species currently used in risk assessment are the ones considered the most sensitive to all pesticides. This approach, which can be considered a good worst case scenario at broad scale, could fail at detail scale where sensitive species living on specific crops could be exposed to pesticides.
In this work a realistic approach has been developed to correlate the avian population at local level, derived from a monitoring survey, with the land-use (Corine land-cover) and the relative pesticide treatments.
A GIS analysis was performed to identify peculiar combinations crop/bird species in order to determine which species is most likely to be exposed to a specific active substance.
The area considered was the North of Italy characterised by an intensive agriculture and a consequently high load of pesticides.
These results could be an useful tool to address the uncertainty associated to the bird risk assessment.

TU 313
Toxicity of sludge water treatment station for aquatic invertebrates
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The water treatment stations (WTP) were worldwide operating in order to treat and deliver potable water from untreated river water. A large quantity of sludge is generated in the water treatment process, which contains suspended solids, bacteria, heavy metals and others toxic substances present in the untreated water. The amount of sludge generated in treatment is directly proportional to the amount of treated water, a WTP that treat 1500 L/s of water generates approximately one ton of sludge/day, which returns to the origin river. The aim of the present study was to evaluation the sludge toxic potential located in Piracicaba, São Paulo, Brazil. The samples were collected in two phases of treatment called: flocculation (first phase of treatment) and decanter (second phase), in July and October 2009 and September 2011. The toxicity tests were carried out with the bioindicators: *Pseudokirchneriella subcapitata* and *Hydra attenuata*, and the dates were compared to the physical and chemical determinations, such as: pH, electrical conductivity and dissolved oxygen (DO). For P. subcapitata and H. attenuata tests were prepared an elutriate solution mixing the sample with distilled water (1:4 ratio). The elutriate samples was used as 100% and diluted to 75, 50, 25, 12.5 and 6.25% with specific culture medium. The samples collected in October 2009 were slightly more toxic for *P. subcapitata* and *H. attenuata*. Both samples were high toxicity for *P. subcapitata*, showed IC_{50} of 11%. However for *H. attenuata* showed EC_{50} of 15.4 and 19.1% and LC_{50} of 17.7 and 64.6% (flocculation and decanter, respectively) which correlated with the highest electrical conductivity determinations (660 and 404 mS cm^{-1}) and DO (0.79 and 3.87 mg L^{-1}). The proximity of the EC_{50} and LC_{50} results for *H. attenuata* indicate the predominance of acute affects, or lethal stages (tulip and crumbled), showing the effective power of the toxic sludge, mainly the one collected in the flocculation tank. It´s possible to conclude that both species tested were adequate to characterize the toxicity of the sludge generated by WTP. Both sludge were very toxic to these organisms and the toxicities were strictly correlated with the increase of electric conductivity, the high concentration of heavy metals and the lowest concentration dissolved oxygen in elutriate.

TU 314
Toxicity of copper on the freshwater snail Physa acuta: reproduction output and biomarkers of effect
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Copper constantly enters aquatic environments through several paths such as industrial effluents, agriculture runoffs and domestic wastewaters. Trace amounts of copper are needed for metabolic and biochemical processes in several organisms but, like for every chemical compound, in excess it becomes toxic. This study aimed to assess effects on reproduction and detect stress responses using biomarkers of effect in the freshwater pulmonate snail *Physa acuta* exposed to copper sulfate in laboratory conditions. A 14/28 days chronic test was conducted using adult snails in 5 different copper concentrations showing that reproduction was impaired as the concentrations increased. Biomarkers activity (e.g. AChE, GST and CAT) and energy reserves were determined and correlated with reproduction. As expected the energy cost allocated to the detoxification process was one of the reproduction impairment causes.

Aquatic snails, like *Physa acuta*, have proved to be good test organisms to assess the presence of chemicals in the environment as they have proven to be very sensitive to exposure.

TU 315
Inhibition of the fatty acid synthesis in chlorophytes by triclosan - a metabolomics approach
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Triclosan is an antimicrobial substance which is used in many pharmaceutical and personal care products such as soaps, toothpaste and mouthwash. -Because of this broad application it has been detected in streams, seawater and sediments in concentrations up to 0.007 µmol L^{-1} [Singer et al., Environ. Sci. Technol., 2002]. In a prior study Franz et al. [Aquatic Toxicology, 2008] observed that chlorophytes are one of the most sensitive organisms affected by triclosan in the range of environmental concentrations.
This raises the question of metabolic pathways affected by triclosan in chlorophytes. We used an established metabolic approach to analyse exposed green algae Scenedesmus vacuolatus [Kluender et al., Metabolomics, 2009]. Therefore we exposed a synchronised culture of S. vacuolatus with different concentrations of triclosan (range from 0.002 to 0.071 µmol L^{-1}) for 14 hours to derive concentration-depended changes in metabolites and to be able to compare them to phenotypic observations (inhibition of cell growth and photosynthetic activity). After harvest and derivatization hydrophilic and lipophilic metabolite extracts were analysed with GC-MS and evaluated by multivariate

statistics (PCA). Two concentration dependent response patterns could be identified. Metabolites from the hydrophilic phase showed comparable sensitivity as phenotypic observations (EC50 algal growth: 0.02 µmol L^{-1}). In contrast the lipophilic metabolites of the green algae were affected significantly at the lowest tested concentration. Identification of lipophilic metabolites with the NIST and Golm library for GC-MS data revealed many fatty acids (e.g. hexadecanoic acid, oleic acid, octadecanoic acid) changed at low concentrations. These findings will be discussed in the perspective of the connectivity of response pathways to the known mode-of-action of the toxicant in bacteria, the inhibition of the Enoyl-ACP-reductase during the fatty acid elongation [McMurry et al., Nature, 1998].

TU 316

Morphometric and biochemical evaluation in gills of Lepomis gibbosus, after acute exposure to several xenobiotics (pesticides, detergents and pharmaceuticals)

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In recent decades, research and awareness of issues related to environmental exposure to anthropogenic xenobiotics has increased. These compounds can affect non-target organisms, altering their physiology, and consequently endangering the balance of ecosystems. Some studies reported the evaluation of morphometric and biochemical changes in fishes, after exposure to several compounds, including pesticides, detergents and pharmaceuticals, which can be quantified using appropriate analytical tools. The gills are important organs in fish since they perform numerous functions. Their multifunctionality, the vast surface of exposure to the external media and their location in the fish body, justify their importance as key organs for the direct action of pollutants in the aquatic environment. This work aimed to assess biochemical changes in gills, in terms of oxidative stress/phase II conjugation isoenzymes glutathione S-transferase - (GSTs) and gill morphometric analysis (secondary lamellar length -SLL and width - SLW, interlamellar distance - ID, and basal epithelial thickness - BET), after acute exposure to the pesticide chlorfenvinphos, the detergent SDS and anticholinesterasic pharmaceuticals (neostigmine and pyridostigmine). The results of this study revealed that only neostigmine significantly decreased the activity of GSTs at concentrations of 1 and 100 mg/L. Considering the morphometric analysis of the gills, the data obtained showed that chlorfenvinphos increased values of BET and decreased SLL at a concentration of 0.75 mg/L. SDS increased values of SLL in fish exposed to a concentration of 3.9 mg/L. The drug neostigmine was responsible for an increase in SLL values at the highest tested concentration, and pyridostigmine increased SLW values at the concentration of 100 mg/L. The potential action of neostigmine in exposed fish, with inhibition of GSTs activity, is a noteworthy result, since it establishes a reduction in the effectiveness of conjugation and elimination of other xenobiotics, enhancing toxicity. Gill morphometrical indices showed evidence of a surface respiratory disturbance as result of exposure to several xenobiotics, which could be advantageous for fish by reducing toxicant absorption.

TU 317

Oxidative stress and higher level effect of engineered nanomaterials in the nematode Caenohabditis elegans : functional toxicogenomic approach

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Toxicogenomics have been increasingly used in ecotoxicology but the challenges of these technologies are infereing biological importance from the data. To deal with this problem, integrated pathway and network analysis are being applied as they give a better understanding of biological effects at multiple levels. This systemic approach is particulary interesting for investigation of chemicals of which mode of actions are less characterized, such as, nanomaterials. Despite the recent increase in research on the toxicity of engineered nanomaterials (ENM) serious deficiencies in the knowledge relating to this area still exist, especially, the relationship between higher level effect and the mechanism of toxicity. Current mechanistic studies on ENM have reported oxidative stress as one of the most important mechanism of toxicity. In this study, to understand the relationship between higher level effect of ENM and mechanism of toxicity, functional toxicogenomic analysis was conducted in the nematod *Caenohabditis elegans*. Survival and reproduction were first examined in worms exposed to silver nanoparticles (AgNPs) and multi-wall carbon nanotube (MWCNT). Subsequently, mechanism of toxicity was investigated using microarray followed by gene ontology (GO) and pathway analysis. Finally higher level effect of altered genes and pathways selected as mechanisms of toxicity was investigated using functional genomics tools such as, loss of function mutant or RNAi of genes involved in these pathways. Their responses were compared with those of wildtypes.

TU 318

Biomarkers evaluation on the pearl oyster Pteria sterna (Gould, 1851) in The Ensenada de la Paz B.C.S. México

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The pearl oyster is an important resource in the State of Baja California Sur Mexico, due to a lack of studies related to to determine their response to xenobiotics in this study an evaluation of 4 biomarkers: respiration rate, O:N index, oxidative stress and AchE activity were carried out in the gill tissue of pearl oyster, from the cultivation vivero, to detect presence of toxic and genotoxic substances, and evaluating the use this biomarkers as reliable tool in environmental biomonitoring studies. Adults oysters (5.15 ± 0.58 cm) were collected in winter (feb 2010) and summer (sep 2010) in the bed, located near to the Pichilingue harbour. Samples tissue gill, was disintegrated; oxidative stress was determined as malondialdehide (MDA) concentrations; and AchE inhibition by means of the Ellman technique. The results indicated that not significant differences in index O: N, the degree of oxidative stress and AchE activity among the organisms collected in summer and winter were detected, but significant differences exist in the oxidative stress grade and AchE inhibition being the pearl oyster collected in Pichilingue and the organisms exposed to toxic metals. It is evident that these biomarkers are a good tool in environmental biomonitoring studies.

TU 319

Physiological and biochemical biomarkers in the catarina scallop Argopecten ventricosus (Sowerby, 1842), as tools to Monitor in the Ensenada de la Paz B.C.S., México.

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The catarina scallop constitutes an important fishing resource in B.C.S., Mexico state, due their production to decreased 86%, in this paper, an evaluation of 5 biomarkers: respiration rate, feeding rate, O: N index, oxidative stress and genetic damage in the scallops, obtained from the in the cultivation nursery of UABCS and CICIMAR was done, to detect the presence of compounds with toxic and genotoxic effects and evaluate the use of these biomarkers as a reliable tool in environmental monitoring studies. Adult clams were collected (15 to 20 organisms) (5.0 ± 0.5 cm) during the summer and winter for 3 years for the evaluation of biomarkers. The biomarker data were integrated for calculation of BRI (Biomarker response index) to establish the biological health of the organisms collected at each site. Significant differences in feeding rate, index O: N, the degree of oxidative stress and genetic damage among the organisms collected in summer and winter were detected. The organisms collected in summer had higher levels of lipid peroxidation (42.7 nM MDA g-1) and a higher degree of cells with genetic damage (24%) compared with the results obtained in winter (9.07 nM MDA g-1 and 15.8% damaged cells). According to the mean values of BRI most degraded site was CICIMAR-IPN. The previous results are consistent with the levels of pollutants recorded in areas where clams were collected. It is clear that the battery of biomarkers used is a useful tool for environmental biomonitoring studies.

TU 320

Relationship between exposure duration, mortality, bioaccumulation and physiological parameters in glochidia (larvae) and juvenile freshwater mussels exposed to copper

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Species richness and population density of freshwater mussels Unionidae have declined substantively throughout North America. Surveys have revealed that environmental pollution was one of the top five stressors to these species and that the early life stages are one of the most sensitive to copper pollution. In this respect, this study aimed to characterize the acute (96 h) and chronic (28 days) sensitivity in newly released glochidia larvae (L. cardium) and 6 months old juvenile (L. siliquoidea) freshwater mussels exposed to copper and understand the toxic mechanism(s) of action. Dissolved EC50 and EC20 for glochidia larvae was 22.7 and 9.7 µg Cu/L, respectively. These values indicate that L. cardium are protected by the BLM-based acute WQC 2.3 µg Cu/L but under protected by the hardness-based WQC 11.3 µg Cu/L. Glochidia copper uptake and accumulation indicate that there is a bioadsorption process based on physicochemical interactions between metal and functional groups of cell membrane which can cause damage at the cell surface. Our results also showed a decrease in whole body sodium concentration. Moreover, we observed an increase in the amount of Reactive Oxygen Species followed by a decrease in Total Oxyradical Scavenging Capacity and an increase in Lipid Peroxidation, characterizing a situation of oxidative stress. For the chronic toxicity test with juvenile mussels, the EC50 was 10.3 µg Cu/L and when they were exposed to 2 µg Cu/L, 21.3 % mortality was observed. The survival data indicated that L. siliquoidea is barely protected by the BLM-based chronic WQC of 1.45 µg Cu/L but significantly under protected by the hardness-based WQC 7.8 µg Cu/L. Results from copper bioaccumulation suggesting that copper toxicity is not associated with its bioaccumulation. Most striking in the chronic experiment was the reduction of whole body sodium content and inhibition of Na+ K+ ATPase, indicating that copper toxicity for mussels chronically exposed is a consequence of an ionoregulatory disturbance. In contrast to the acute experiment, redox parameters did not show significant differences between control and copper exposed mussels, but a lipid peroxidation could be observed in both tests acute an chronic. Take into account our results, mechanisms of copper acute toxicity for these sensitive mussels seems to be different to the chronic ones. This difference can be related to the time of exposure and/or to physiological specific aspects of different life stages.

TU 321

Cryptic lineages - are they comparable in their sensitivity towards chemical stress?

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The term “cryptic lineages”covers taxa that exhibit genetic differences but an at least superficial morphological equality and the respective specimens thus are misleadingly classified as a single nominal species. Although a well-known concept in evolutionary genetics since several decades, its relevance in an ecotoxicological context has only poorly been investigated. However, there is an urgent need for an ecotoxicological assessment of cryptic complexes as the genetic differentiation between lineages often reaches magnitudes similar to those between species. Thus, deviations regarding behavioural and physiological endpoints within cryptic lineage complexes are conceivable.

Accordingly, the present study investigated two cryptic *Gammarus fossarum* lineages - namely type A and type B - for differences in their sensitivity towards chemical stress. Two size classes of each cryptic lineage type were exposed to different concentrations of the fungicide tebuconazole or the insecticide thiacloprid in six independent experiments. The feeding rate on leaf discs was determined after seven days of exposure as a measure of sensitivity.
The subsequently conducted meta-analysis revealed a statistically significant higher overall sensitivity of the cryptic lineage type A compared to type B ($n=16$; $p<0.0001$), by combining the results of all bioassays. Discrete meta-analyses for each of the substances tested, showed a similar tendency, however, results were statistically significant only for tebuconazole ($n=8$; $p<0.0001$). This deviation in sensitivity between lineages is new due to the minor consideration of the cryptic lineages concept in the field of ecotoxicology, however, mechanistically yet not explainable. Hence, further studies should address the sensitivity to other stressors, by also considering ecological aspects like the physiological fitness, of a broader range of cryptic complexes. Nonetheless, genetically distinct lineages may confound the results of ecotoxicological tests, as indicated in the present study, and should thus be interpreted with caution.

TU 322
Status of PAHs in greater Johannesburg area and possible sources
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The presentation gives an overview of the status of the presence and levels of PAHs in greater Johannesburg area. Although many studies of persistent organic pollutants such as pesticides have been studied in South Africa, the study on PAHs is still having a lot of gaps. The presentation gives an overview of modern extraction techniques that have used for the extraction of the PAHs in water and sediments in rivers and dams in greater Johannesburg area. The common PAHs found and their concentration levels are presented. The levels of PAHs in water are compared to those in sediments. Detailed discussion of the possible sources of PAHs is given and how these sources impacts aquatic ecosystems in the studied area. Finally, the overview of PAH status in South Africa urban environment is given.

TU 323
Linking transcriptome to phenotype: response of a mouse liver cell line to Benzo(a)pyrene
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The aim of the systems biology initiative, “From contaminant molecules to cellular response: system quantification and predictive model development”, is to build a model of interactions between cells and the toxic chemical Benzo-a-Pyrene (BaP). As part of this initiative, we aim to identify the regulation of genes by BaP in a time and concentration-dependent manner and relate the regulation to cellular distribution of BaP and the physiologic or toxicologic response. Exposure to BaP has been linked to carcinogenic, immunosuppressive and pro-inflammatory effects but there is still limited knowledge on BaP interactions with cells on a systems level. The well characterized Hepa1c1c7 was chosen as a cellular model to identify cell-BaP interactions and advance mechanistic understanding and predictability of the multiple toxic responses. In a first step, two concentrations (50 nM and 5 µM) and four time points (2, 4, 12 and 24 h) were used for comprehensive transcriptome analysis. The results show a small number of genes being regulated at the early time points for both concentrations and a high number of genes being regulated at later time points for the high concentration. Genes like Cyp1a1, Tiparp and Nqo1 are regulated early for both concentrations whereas Aldh3a1, well known to act against oxidative stress, is only regulated at the higher concentration. Chemical toxicity, cell viability, detection of ROS formation and lipid droplets were determined. There was a significant reduction of the cell viability but only after 48 hours at 5 µM BaP. Hypoxia and oxidative stress is one process network strongly regulated after 12 hours of BaP exposure. Using H2DCFDA staining, ROS production is already observed after 4 hours of exposure to high BaP whereas no response is observed for the low BaP concentration. However, at 24 hours, ROS appears to be produced even for the low BaP concentration. Gene response after 12 hours of 5 uM BaP exposure moreover shows genes related to lipid metabolisms. Indeed, lipid body staining using nile red revealed significant changes in lipid droplet morphology already after 4 hours. We conclude that the low BaP concentration induces a small gene response, that, together with biochemical defense mechanisms, helps the cell to return to its initial state. However, the high concentration causes strong pathway regulation and cellular dysfunction accompanied by early ROS production and Lipid droplet modification.

TU 324
Insights into the effects of silver by transcriptomics and proteomics in Chlamydomonas reinhardtii
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Ecotoxicology aims to identify effects that toxicants elicit upon the environment. A major challenge is to understand the toxic mechanisms at a molecular level and how these relate to functional changes at the organism and population level. Integration across various levels of biological organization, molecular, cellular and organismal, is required to draw conclusions on impacts in populations, communities and ecosystems (1).
Our research deals with the analyses of silver induced alterations in gene and protein expression in the algae Chlamydomonas reinhardtii and anchoring of these changes to specific phenotypes. This study is multidisciplinary and involves conventional toxicology analysis, transcriptomics, proteomics, metabolomics and bioinformatics. The transcriptome of silver exposed algae were analysed using C. reinhardtii whole genome microarray. The global protein profile of silver exposed C. reinhardtii (5h exposure time point) was done by Multidimensional Protein Identification Technology (MudPIT) which consists of 2D-LC and mass spectroscopy. The transcriptome analysis determined that the most significantly regulated transcripts are those of metabolic pathways of photosynthesis, tetrapyrolle synthesis, mitochondrial electron transport, protein transport and oxidative stress response. Similarly, the proteome profile showed these biological processes being significantly regulated indicating that silver affects key functional pathways in C. reinhardtii. The regulation of several transcripts/proteins involved in the photosynthesis and ATP synthesis could be corraborated with inhibition of photosynthesis and total ATP content at the physiological level. Also,the oxidative stress response could be linked to the physiological endpoint of lipid membrane peroxidation and synthesis of storage lipid bodies. Global analysis of the transcriptome and proteome not only gives an insight into the mechanism of toxicity of silver but also, by linking them to ecologically relevant phenotypes, a better ability to predict enviromental risk.

TU 325
An integrative approach to understanding the response of Caenorhabditis elegans to valproate exposure
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C.elegans has been extensively used to study the effects of chemical exposure, aging, pathogenicity and reproductive development. With a well annotated genome, well characterized cell lineage maps, ease of making knock-outs, availability of mutants and short life span, it is a good model system to study the effect of chemical exposure on biological systems. In this study, wild type N2 C.elegans were treated with sodium valproate, a known teratogen and a histone deacetylase inhibitor used as a first line drug in epilepsy, manic depression, bipolar disorder and schizophrenia. Computational biology and omics technology was utilized to develop gene signatures associated with valproate exposure. We employed a systems biology approach by mapping transcriptomic data to known biological pathways to identify possible pathways affected by valproate treatment. The network models generated with the genes significantly associted with valproate indicates an overall downregulation of nuclear proteins. Metabolite signatures associated with valproate treatment were identified by Nuclear Magnetic Resonance (NMR) spectroscopy on formalin fixed worms.
Functional analysis was done using the DAVID gene ontology web tool. Genes were mapped to known pathways using Ingenuity Pathway Analysis software (Ingenuity® Systems, www.ingenuity.com). Networks derived from the genes highly correlated to valproate exposure were analysed by the same software. Metabolomic analysis was performed on the aqueous fraction of methanol:chloroform extracted samples on Brucker Avance 500MHz spectrometer at HWB-NMR Center, University of Birmingham. C.elegans were fixed prior to extraction, by adding 37% formalin directly on to plates.
Treatment with valproate resulted in an increase in lipid level and a decrease in egg laying in a dose dependent manner. The genes with positively correlated expression to valproate were enriched with functional terms such as structural molecule activity (cuticle/collagen), detoxification, phosphorylation and lipid metabolism. Genes related to embroyonic/larval development/reproductive development, cell cycle/growth, transcription and chromosome organization are negatively correlated to valproate levels. This could be mainly due to the down regulation of the components of the histone deacetylase complex, DNA replication initiation complex, spliceosome and some translation initiation factors by valproate.

TU 326
Development of a resource for the collection, analysis and integration of ecotoxicological-related high throughput data
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In environment, the presence of chemical pollutants or climate changes can induce organism responses at the cellular and molecular level. Beside more traditional analysis system like evaluation of biomarkers, researchers can nowadays benefit of high throughput technologies (i.e. microarray, deep sequencing, 2-DE) that allow very large scale investigation. Data deriving from these technologies offer snapshots of the changes induced by the presence of environmental variations.
Management, analysis and interpretation of the massive data obtained from high throughput technologies can be quite challenging and requires bioinformatics skills still beyond the possibilities of many laboratories focused on “wet biology”. Moreover all these results are poorly informative if considered separately and do not offer an exhaustive overview of the real cellular response and of changes in gene expression. Indeed they do not consider the relation between mRNA level, protein concentration and metabolite levels which are known to poorly correlate. Finally it would be very useful to compare the biological effects induced by the same environmental stress on different organisms through comparative studies.
Here we propose a web based system that works as a centralized resource for the collection, analysis and integration of ecotoxicology related high throughput data. ‘Aggregated’ information is accessible by means of simple web based data mining tools and is supported by effective and simple graphic representations to facilitate the understanding of biological response mechanisms.
We have benchmarked our platform with data obtained by high throughput analysis techniques- microarray and 2-DE - applied on the social amoebae Dictyostelium discoideum after treatment with several doses of mercury.
We show the advantages of using our system which allows the comparison of the results obtained from the two techniques, effective tools for the visual inspection of data and intergrates data from external resources such as specialized databases (ie. genomic databases, metabolic pathways, Gene Ontology).

TU 329

On the appropriateness of using the common mixture toxicity models CA and RA on species sensitivity distributions: a theoretical approach

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Environmental species are not only exposed to single substances but typically to mixtures. Therefore it is of great importance to assess the ecological risk not only substance by substance but also for mixtures of compounds. Two models are generally used to predict mixture toxicity: the concentration addition (CA) (or dose addition) and the response addition (RA) (or independent action) models. Their adequacy to predict mixture toxicity of similarly acting compounds (CA) and dissimilarly acting compounds (RA) for single species was already shown during the last decades. To assess the risk of chemical mixtures to ecosystems, these two models have also been proposed to be applied directly on species sensitivity distribution (SSD) curves to derive a multi substances potentially affected fraction of species (msPAF). Usually, this is done in two steps: (i) the CA model is applied on individual SSDs of similarly acting compounds and then (ii) the RA model is applied on the predicted SSDs by CA between groups of similarly acting compound. However, as mentioned above the validity of mixture models were shown only for single species when applied on single dose-response curves and not on multiple species SSDs. For a validation of this approach, one should therefore first apply the mixture models for each species, then construct the mixture SSD to calculate the msPAF and compare it with a msPAF derived by applying the mixture models directly on the SSDs. This approach is however rarely done because the information on the whole dose-response curves of each single species for all substances is needed and this information is poorly available. .
In this study, we highlight the difference between the two methodologies (SSD combination with CA/RA models or single species mixture assessment followed by SSD construction) to derive a msPAF. To compare the two methods we calculate msPAF with different cases, varying the EC50s and the slopes of the individual dose-response curves.
The results show that the predictions with CA are quite similar between the two methods and small differences can be explained by the goodness of fit of SSDs and the species tolerance correlation among substances. However, the application of RA may result in a differing msPAF between the two methods. Finally, we define cases in which the two methodologies give similar results and for which the use of CA and RA directly on SSDs to predict the risk of mixture compounds is appropriate.

TU 330

Estimating inter-individual sensitivity from survival data using a mechanistic model

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In the literature, there are two main alternatives to model mechanistically dose-survival relationship in ecotoxicity tests. Effects are related to a concentration of concern, for instance body concentration, and either individuals have different concentration thresholds for death, or individuals have equal probability to show an effect, with dying organisms just being the "unlucky"ones. A general framework to unify both approaches has recently been proposed but only special cases could be confronted to actual data. We used such a unified model to analyse four datasets. We showed the possibility to estimate properly the toxicity parameters together with inter-organisms differences of sensitivity for at least one of these parameters (here the threshold for effect). For all datasets, the model we propose outperformed the standard approach without accounting for differences of sensitivity. More accurate parameters' estimations would be obtained through the incorporation of prior knowledge, in particular relative to background mortality. We also pointed that the standard threshold estimate is close to the medium value of the distribution (which would induce the death of half of the organisms), and that more relevant parameters; such as the concentration protecting 95% of the population would be 2 to 4 times inferior to this standard threshold. We believe that our approach is easy to understand and to apply by non-experts in modeling. Moreover, it permits to relate two fundamental concepts in understanding the response in ecotoxicity tests: the link between internal concentration and effects and the inter-individual variability regarding sensitivity.

TU 331

Incorporating data quality scores and acute-to-chronic ratios in the construction of SSDs

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The species sensitivity distribution (SSD) approach is in principle an alternative to the assessment factor approach for PNEC calculation, However, due to requirements on data quantity, its use is currently restrained to a small number of compounds. One central problem is that commonly many available data are discarded from the analysis for reason of limited validity, endpoint (exclusion of EC_{50} type data) and exposure time (exclusion of acute date).
The objective of the present study was to assess how the SSD approach can be extended to the use of the entire data sets of complex heterogeneous ecotoxicological information (data of different data quality, acute and chronic values, EC_{50}, EC_x and NOEC values). More precisely, we aimed to provide a method which provides reliable and robust PNEC assessment, which is applicable to wider area of compounds.
For this purpose, we replaced the dichotomic classification of data as either valid or not valid by a system of quality scores which integrate on the inherent experimental and statistical reliability of data and on their biological relevance (organism and endpoint). This system serves as weighting factors of individual data in the SSD analysis. Furthermore, we studied different options of how to incorporate acute values. Different options (use of probabilistic Acute-to-Chronic ratios and weighting) for data treatment were integrated in a tool, which allows a generation of SSD functions and estimate uncertainties.
As test cases, we calculated the standard SSD function (based on chronic NOECs only) for several real ecotoxicological data sets on compounds fulfilling the strict TGD/REACH requirements for SSD application. Subsequently, we applied the developed method using weighting scores and acute to chronic extrapolation to the entire data set and bootstrap subsamples. SSD function obtained for subsamples of the entire data set using different options for data treatment were compared to the SSD based on chronic NOECs. Influences of different options for data treatment and sample size will be discussed with respect to the robustness and reliability of the SSD function and the associated HC_5 value.

TU 332

Investigating the replacement of NOEC and LOEC values with regression-based ECx values

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Data from chronic toxicity tests have traditionally been analysed by hypothesis test based statistical analysis to obtain a No Observed Effect Concentration (NOEC) and a Lowest Observed Effect Concentration (LOEC). Although a firmly entrenched paradigm, much recent debate has questioned whether these methods are the most appropriate way to analyse data from eco-toxicology studies. As part of this debate the use of ECx values and associated confidence intervals, determined by regression analysis and other modelling techniques, have been investigated by the OECD and others as possible replacement or complementary endpoints and are now included as a requirement in some test guidelines.
From the analysis of a number of data sets, the work described has shown that it is possible to apply a number of different static regression models to results arising from ecotoxicity tests, and to derive ECx values. The work indicates that, although not consistent for all datasets examined, it is possible to derive ECx values broadly similar to the respective NOEC and LOEC values.
However, a number of issues remain which will be of importance when considering the possible replacement of NOEC and LOEC values with ECx values, including experimental design. It is thought that current test designs may not necessarily be suitable for data analysis using regression based methods, and that modifications to current protocols may be required to ensure that regression analysis of test data yields reliable and significant results.
The work described has applied regression analysis to a number of simulated data sets which were developed according to differing test designs. This has shown that the experimental design of an ecotoxicity study can have implications for the results obtained by statistical analysis using regression techniques. In particular, both the estimated ECx values and the associated confidence intervals have been shown to be impacted by experimental design. This could have implications for both the ethical and practical aspects of ecotoxicological testing, when seeking an optimum test design.

TU 333

Statistical approach to select soil bioindicators for soil monitoring, risk assessment and soil characterization. Results from the French national programme 'Bioindicators'

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The Random Forests statistical approach (Breiman, 2001), is used to classify, predict and select the best set of explanatory variables in large fields of research. Yet, only few applications were made in environmental research, especially in soil ecotoxicology.
The analytical strategy of the French "Bioindicators Programme"(2006-2012) is to (i) measure a large number of bioindicators in contrasted pedo-geo-climatic contexts covering French area, using similar sampling protocols, (ii) manage high numbers of biological and meta-data in a database enhancing data analysis, (iii) develop a data mining of collected data during two years, with the help of a biostatistician team. The main objective is to develop a generalised method for selecting relevant bioindicators with regard to the targets (characterisation, monitoring, risks[3DOTS]), that may assess risks of soil contamination to ecosystems.
More than 170 biological variables are screened, including microorganisms (e.g. biomasses of fungi and bacterial communities, bacterial diversity, enzymatic activities), mesofauna (e.g. nematodes and collembola), macrofauna (e.g. earthworm at community levels and metal accumulation in snails). Such bioindicator variables were measured in soil surface horizons, at 13 sites, producing 47 different contexts in terms of land use historic and origin of contamination (PAH, ETM, pesticides).
As a first step of statistical analyses, our results showed the importance of correlations between biological variables in intra and inter-groups. Then discriminated analyses using Random forests revealed sets of relevant indicators for land use (crops, pastures, woods and forests), levels of metal contamination (high, medium and low) as well as

organic contaminations.

TU 334
Advanced non-linear regression methods for concentration-response curves in R
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A recent editorial in IEAM has suggested to ban the use of traditional no-observed-effect levels (NOELs) and lowest-observed-effect levels (LOELs) as basis for risk assessment and decision making, and instead use curve-fitting for estimation of concentration-response relationships. This should motivate an evaluation of current concentration-response modelling approaches in ecotoxicology, and adoption of new methodologies for curve-fitting that are used in other fields of environmental science. The European Water Framework Directive (WFD) has triggered much research on non-linear relationships between physico-chemical stressors and ecological responses during the last decade. For example, thresholds in stressor-response relationships are particularly relevant for assessment of ecological status of water bodies. Basic statistical methods such as linear regression or ANOVA are not suitable for estimating such relationships. The response may also be more complicated than e.g. a simple sigmoid curve, therefore flexible regression methods have been used for exploring the shape of the response curve without a priori assumptions. Moreover, we may be interested in estimating an extreme part of the response rather than the average. In this presentation, I give examples of ecological responses to eutrophication and acidification stress in lakes. The ecological responses are univariate indices representing community composition of phytoplankton, macrophytes, macroinvertebrates and fish. Building upon basic linear regression, I describe three statistical 'tricks' which enable more flexible curve-fitting: (1) Using a non-linear link to the response variable (generalised linear model); (2) Using a piece-wise linear function for the stressor variable (generalised additive model); (3) Analysing a quantile of the response variable instead of the average (e.g. 90%; quantile regression). All analyses are carried out in the free statistical programming software R.

TU 335
An interfaced R-package to fit SSDs
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Species Sensitivity Distributions (SSDs) rely on the assumption that aquatic species of a community or assemblage differ in their sensitivity to a hazardous chemical. Toxicity values are used as indicators of the sensitivity and are assumed to follow a theoretical distribution accounting for the inter-species variability. A Hazardous Concentration (HC) is then derived using a low-order percentile of the SSD fitted to a limited (and often small) set of toxicity values, the latter being assumed to be a representative sample of species sensitivity among the community to be protected.
The SSD approach is an element of most regulatory guidance documents for environmental risk assessment (e.g. ANZECC, ECHA, USEPA). Yet, the approach to be used is still debated, including: (1) whether parametric or non-parametric methods should be preferred - the applicability of the latter being limited by the small size of toxicity data sets available and (2) in the parametric cases: (2a) the choice of the distribution shape - this is often decided on the basis of mathematical properties, especially on the existence of analytical results, and (2b) the choice of the method for estimating HCs and their confidence interval.
Several softwares have been provided through environmental protection agencies for estimating HCs and their companion uncertainty, such as Burrlioz in Australia and New Zealand, ETX in The Netherlands or Web-ICE in the U.S. Those three softwares have been developed as turn-key tools for the application of regional regulatory recommendations and rely on different choices regarding the three points aforementioned. Besides, it appears that none of these programs allows censored data to be accounted for. For users interested in examining the impact of the methodological choices made in existing softwares (and in the corresponding regional guidance documents), we are currently developing a new and open source tool enabling the comparison of several distributions and also the use of both pointwise and interval-censored data (or a mix of both).

TU 336
A probabilistic model for species sensitivity distributions taking into account inherent uncertainty and variability of effects to estimate environmental risk
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Empa, St. gallen, Switzerland
TU 337
Toxicokinetics-Toxicodynamics survival model, from theory to practice
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Toxicokinetics-toxicodynamics (TKTD) models simulate the time course of processes leading to toxic effects on organisms. Even for an apparently simple endpoint such as survival, a large number of TKTD models exist. They have been reviewed by Jager et al (2011) who proposed a unified theory (GUTS) for TKTD threshold models and the use of a likelihood approach for estimating model parameters. Here, we focus on a TKTD threshold model in the GUTS framework called DEBtox (Dynamic Energy Budgets in toxicology) survival model. This model assumes an effect above a threshold concentration. We examine a simple question: is it possible to estimate parameters of this model by maximum likelihood approach from standard survival data sets?
We analyzed survival data sets of the freshwater invertebrate Daphnia magna exposed during at least 21 days to nine different contaminants. We tried to fit 4 nested DEBtox survival models to each data set by maximizing the log-likelihood function to estimate model parameters.
We illustrated two structural problems linked to the model itself or to the likelihood function. (1) We cannot simplify the model by fixing the natural mortality to 0 even if data did not show natural mortality and (2) because of discontinuities in the log-likelihood cross sections when the threshold parameters is equal to one of the tested concentrations, there are many local minima. This is due to the structure of the threshold model and especially to its non-derivability at the threshold value. We will investigate various solutions to this issue, including adaptation of optimization algorithm and modification of models with fully derivable functions.

TU 338
The IBR revisited: optimization to avoid misuse
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Multibiomarker approaches are widely used for in situ assessment of ecotoxicological effects of contaminants and to understand the relationships (1) between biomarkers and (2) between biomarkers and contamination status of studied sites. Several tools have been proposed to integrate these responses in a single and simple measure. Among them, the Integrated Biomarker Response (IBR) is a promising method that provides both a graphical synthesis of the different biomarker responses and a numeric value that integrates all these responses at once. The IBR is the sum of the area defined by the k biomarkers arranged in a radar diagram. However, in their initial publication, the authors provide two calculation methods: the first one is a complicated formula that works wathever the number of biomarker is, while the second one is a simplified formula that works only when 4 biomarkers are used. The attractiveness for simplicity led to frequent misuse of the IBR (40% of the 38 publications using it). Moreover, the final outcome of the calculation process highly depends on the sequential organisation of the biomarkers. We aim to propose a new procedure to resolve these main problems in the IBR application.
We first go back to trigonometry basics to propose a new formula for the IBR, that is far more simple than the original one. Then we wrote a procedure that creates all the possible circular permutations of k biomarkers. It results on a (k-1)! matrix of IBR value that allows to calculate the mean IBR for a site and to prioritize IBR values among sites in a more confident way.
A case study using this method is presented, based on the results of a survey of 8 sites presenting contrasted levels of sediment contamination. The permutation is performed for a set of 7 biomarkers in the bivalve Dreissena polymorpha, resulting in a matrix of 720 IBR values. It evidenced the variability of the IBR, that is not our main concern. Indeed, the consequence is a different prioritization of contaminants effects depending on the biomarker sequence, that could lead to misunderstanding of contamination consequences on biota. Considering the need of multibiomarker approaches to understand the complexity and the variability of biological responses, our study provides an efficient and robust tool to integrate these data and avoid subjectivity in the final outcome of the method.

TU 339
Statistics Service - a new tool for efficient data analysis
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In this talk we present a newly built statistics tool which allows a highly convenient and efficient data analysis. The tool combines Excel (a Microsoft Office product) and R (a cost-free available statistics programming language) via a specific server.
This Excel-R connection significantly broadens the spectrum of available statistical methods in Excel. Moreover, the user (e.g. lab technician) can easily conduct complex statistical analyses:
The user selects the desired statistical method in Excel after highlighting the input data for the computational analysis. The data is then transferred to the server, the computation starts and the results are automatically transferred back to Excel. Besides the results (e.g. graphical plots) the user can also receive an interpretation help (txt file) of the result output.
In addition to a short introduction of the Statistics Service technical background, we will demonstrate its functionality using examples from the environmental fate modeling for plant protection products, e.g. the analysis of environmental fate data and selection of relevant input parameters to exposure models, in order to provide a general overview of how this tool works.

TU 341

Long term effects of an early exposure to PAHs on zebrafish behavioural responses

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Polycyclic aromatic hydrocarbons (PAH) emission in the environment is constantly increasing with human activity. The goal of this study was to assess long term consequences of fish early exposure to PAH on behaviour. Eggs were collected from AB zebrafish strain and were placed at 4 hours post fertilization (hpf) until 96 hpf in a 3 cm diameter Petri dish containing 3 g reference sediment either plain (control) or spiked with a mixture of Benzo[a]pyrene, Pyrene and Phenanthrene at environmental concentrations based on values measured in the Seine Estuary (France), a highly polluted site. Thereafter, larvae were transferred in clean water and raised until adulthood. Behavioural tests such as locomotion during 24-hrs, sudden dark change challenge and T-maze exploration were performed at adults' stage (F0) and sudden dark change on larvae produced by F0 adults. In adults, during night or dark period, contaminated fish were significantly less active than control fish. A similar observation was made for contaminated F0 in T-maze safe area. Contaminated F1 larvae were more active when light was on than control and less active when the light was off. This study indicates that contamination of zebrafish using environmentally relevant concentration during the very first stage of development with a PAH mixture of 3 molecules induced late behavioural effects measured at the adult stage. Further, effect was transmitted to the next generation and behavioural responses of F1 larvae were different to that of F1 control larvae. Consequences on fish abilities (e.g. predator escape, food search, courtship) will be discussed.

TU 342

Long-term food-exposure to PCB mixtures induces reproductive and behavioural disruptions in zebrafish

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Although the use of polychlorinated biphenyls (PCBs) has been banned for several decades, they are still present in the environment. Field analyses have established correlations between exposure to PCBs and alterations in fish physiology including reproductive function and behaviour. In the present study, we performed a life-cycle exposure using zebrafish and mixtures representative of some environmental situations in terms of doses, composition and containing mainly non dioxin-like congeners. Exposure was performed through diet which is the main contamination route in the wild. We demonstrated a bioaccumulation of PCBs in males and females as well as a maternal transfer to the eggs.

Several reproductive traits were altered after exposure to a PCB-contaminated diet, including a reduction in the number of fertilized eggs per spawn as well as an increase in the number of poorly fertilized spawns. This was related to modifications of ovary histology revealing a decrease of maturing follicles and an increase of atretic follicles in the ovaries of females exposed to PCBs.

In addition, several behavioural traits were monitored. Fish exposed to the highest dose (equivalent to that found in the Seine Estuary) displayed an increased swimming activity during the period at the end of the night. Fish exposed to an intermediate dose (equivalent to that found in the Loire estuary) displayed behavioural disruption analogous to hyperactivity that showed some similarities to a human behavioural deficit syndrome known as attention deficit / hyperactivity disorder (ADHD), which can be observed in humans after exposure to PCBs.

These results indicated that exposure to PCBs mixtures mimicking some environmental situations, can lead to a dramatic reduction in the number of offspring produced by a female over a lifetime and disrupt behaviour. Potential ecological consequences are discussed with regard to reproduction, fitness and survival.

TU 343

Indirect estimation of population-level effect of pollutants based on tolerance evolution and fitness cost of tolerance

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Evolution of tolerance to a pollutant chemical in a natural population of organisms gives an evidence of the examined population to have suffered adverse effect by the chemical because the tolerance evolution of a population is solely brought about by selection pressure that removes sensitive individuals or genotypes from the population. Despite the advantage in using the tolerance evolution as a monitoring tool of chemical pollution in the field, such approach is not relevant for a quantitative impact evaluation because observed differences in the tolerance between a contaminated and an uncontaminated site are rarely connected to the ecological risk that burdens the population.

Applying the theory of evolutionary ecology and quantitative genetics into the tolerance evolution, we attempted to estimate the selective force that is needed to maintain the observed difference in the tolerance between populations. For this aim, we made a heuristic assumption for the evolutionary analysis of tolerance: the strength of tolerance induced by a genotype to a particular compound is positively associated with fitness cost of the tolerance, and the joint action of selection induced by the cost and selection favoring higher tolerance achieves optimization of the total fitness that takes into account the fitness gain by the tolerance and the fitness cost due to the tolerance. The fitness gain by the tolerance means reduced toxicity of a specific exposure concentration in comparison to sensitive individuals, and can be quantified by the life table toxicity data. As a case study of such an approach, we detected inter-population differences of the fenvalerate tolerance among natural populations of a water flea (Daphnia galeata) in Kasumigaura Lake (Japan) and an agricultural reservoir near to this lake. There was a statistically-significant negative association, among isofemale clones, between tolerance values and intrinsic population growth rates under null exposure. With the fitness optimization, we derived an estimate of stationary exposure level of the chemical (all compounds that exhibited co-tolerance with this chemical) as 0.015 ppb, and concluded that the examined population of this species had been suffering the population-level effect that corresponded to 1/4 reduction of the intrinsic population growth rate. The indicated level of the population-level effect may have considerably reduced the probability of persistence of the population.

TU 344

Triclosan toxicity: a multi-generational and demographic assessment using Daphnia magna

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Triclosan is a ubiquitous compound in wastewater treatment plant effluents and biosolids and is commonly detected in surfaces waters throughout North America and Europe. The acute toxicity of triclosan (TCS) toward aquatic organisms has been well studied but information on long-term, low level exposures at environmentally relevant concentrations is lacking. The objective of the present study was to evaluate the toxicity of triclosan in a multi-generational test with Daphnia magna in laboratory exposures. 24-hr old D. magna neonates were exposed to TCS (0.5 to 150 µg/L) in six successive 21-d life cycle tests. Brood randomly collected from 5 replicate beakers within each treatment on day 21 were used to seed the next generation. Survival across the 6 generations exceeded 80% in all treatments across all generations. A slight but non-significant delay in time to first brood (7-9 days) occurred in the 150 µg/L treatment in the first generation but not thereafter. Total offspring produced over 21 d and fecundity (average offspring/adult) decreased significantly at concentrations >50 ug/L (NOEC = 10 ug/L) in the first 3 generations but this difference disappeared in the last three generations indicating increased tolerance to TCS. Reproductive demographic data were used to estimate the intrinsic rate of increase (r) and indicated significant declines in population levels at the three highest treatments based on the first 3 generations but not the last 3 generations. In this study, effects were only observed at TCS concentrations approximately one order of magnitude greater than those found in surface waters, which indicates that the long-term risks of TCS are likely minimal.

TU 345

Assessing the chronic aquatic toxicity of phthalate ester plasticizers

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Phthalate esters are a class of chemicals varying greatly in terms of uses, properties and toxicity. C1 to C4 phthalate esters are used in non-vinyl commercial products and pharmaceuticals. C8 to C10 phthalate esters are additives imparting flexibility to vinyl resins. The purpose of the present study is to assess chronic effects of phthalate esters on aquatic organisms. Studies show that populations of fish and invertebrates may be adversely affected by exposure to C1 to C4 phthalate esters, but are not adversely affected by exposure to C8 or higher phthalate esters. Secondary endpoints, including molecular, biochemical, and/or histological responses to chemical exposure, do not appear to predict effects related to primary endpoints of survival, growth and development, or reproductive fitness. A previously published risk assessment for C1 to C4 phthalate esters demonstrated low risks in North American and Western European surface waters. Risk assessments conducted by authorities in Europe with DEHP, DINP, and DIDP have concluded no risks to aquatic organisms due to aqueous solubility constraints, low expected surface water concentrations, and metabolic biotransformation capabilities of aquatic organisms. Important chronic aquatic toxicity studies that have included transgenerational exposure have been conducted since those assessments and are presented here. The data from the present studies, support the earlier risk assessment conclusions. The data also provide further support for a narcosis-related aqueous solubility cutoff at approximately C6 and higher phthalate esters. Finally, for the C1 to C4 phthalate esters, the present study shows that secondary endpoints (e.g., molecular, biochemical, and/or histopathology) to date, provide limited benefit in practical ecological risk assessment of phthalate esters to aquatic species.

TU 346

Effects of land use and pesticide exposure on hatching characteristics of Daphnia magna ephippia

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Daphnia is a well established model organism and standard test species in ecotoxicology. *D. magna* reproduces by cyclical parthenogenesis, where environmental cues associated with unfavourable conditions trigger sexual reproduction. In permanent and temporary standing waters, sexually produced encapsulated dormant eggs (ephippia), build up over the years to form a dormant resting egg bank, which is important for long-term survival of the population. Each year a fraction of the dormant eggs hatch from the sediment. Through this benthic-pelagic coupling, structure and function of resting egg banks have important consequences for ecological and evolutionary dynamics of the active populations and communities. To date however, almost no information is available on the effects of pollution on these dormant egg banks.
Coors et al (2009) used land use patterns as a proxy for historical and current pesticide contamination and demonstrated that the tolerance of *D. magna* populations for the pesticide carbaryl was positively correlated with land use intensity of the area around the ponds. As in standard ecotoxicity tests, these experiments were performed for 48 hours with *D. magna* neonates less than 24 hours old.
In this study we investigate the effects of land use intensity and pesticide exposure on hatching characteristics of *D. magna* ephippia from natural populations. Therefore we sampled dormant egg banks of ponds from different regions in Belgium, in landscapes with varying degrees of agricultural activity (from natural areas to areas with intensive agriculture). In the laboratory hatching experiments were performed, looking specifically at differences in hatching characteristics of *D. magna* ephippia under optimal conditions and after pesticide exposure in relation to historical pesticide exposure.

TU 347

Is there a potential for wild Daphnia magna populations to undergo selection at conventionally derived no observed effect concentrations of chemicals?

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The genetic variability within a population exposed to stress governs the micro-evolutionary potential of that population to undergo selection and adapt to the stress. However, adaptation caused by natural selection will by definition reduce overall genetic diversity, a process termed genetic erosion. This may result in a cost of tolerance, whereby populations evolved under a certain chemical stressor, may become less tolerant to novel stressors. It is hypothesized that a population under stress exhibits an increase in genetic variation for life history traits relative to a control population. We used the genetic coefficient of variation (CV_G) and the broad sense heritability (H_2) as measures for genetic variability to assess the potential for micro-evolutionary responses in wild populations exposed to copper and zinc, as model toxicants. Life-table experiments with concentrations of copper and zinc close to the conventionally derived geometric mean NOEC values (50 µg Cu/L, 428 µg Zn/L) were conducted with 8 *Daphnia magna* populations collected from lakes and ponds across Belgium, with non-elevated Cu and Zn levels. Additional full dose-response experiments were carried out with the most and least sensitive populations. Our results indicate that several fitness traits have the potential to respond to natural selection and genetically adapt, but that this depends strongly on the population and the exposure treatment considered. In long-term multi-generational exposures this may result in shifts in genotype frequencies and reduction of genetic diversity.

TU 348

Evolutionary consequences of the loss of genetic diversity in C. riparius populations - Introducing the MIDGE project

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Chronic pollution has been shown to decrease genetic variation in populations of several species alongside with adverse effects on the physiology of organisms. This loss of genetic diversity may reduce the potential of populations to adapt to changing environments.
Ecotoxicological studies need to consider not only short term effects of pollution, such as changes in life history traits of organisms, but also its long term effects, such as genetic erosion. This genetic erosion can lead to the loss of evolutionary potential and thus it is also of crucial importance to assess the evolutionary consequences of changes in genetic variability.
There is an urge for evolutionary toxicology studies because only an integrated approach linking contamination, genetic variability, life-history responses and fitness costs associated with genetic adaptation can lead to a more reliable prediction of the anthropogenic impacts on biodiversity.
In the MIDGE project aims to study the **MI**croevolutionary **D**ynamics and **G**enetic **E**rosion in pollution-affected *Chironomus* populations. More specifically we are investigating the genetic variability of *C. riparius* populations from unpolluted and contaminated sites using mitochondrial sequence variation and nuclear microsatellite analyses and we intend to address three main questions:
- Does chronic pollution affect genetic variability of *C. riparius* populations in the field?
- Are *C. riparius* populations in contaminated areas adapted to pollution exposure?
- What are the evolutionary consequences of this altered genetic variability due to pollution in terms of fitness costs?

Measures of genetic erosion have recently been proposed as the ultimate biomarker of effect and by focusing on effects of contaminants on genetic variability in natural populations we aim to aid in developing new bio-monitoring approaches and provide advanced scientific basis for integrative ecological risk assessment methodologies which are essential for effective environmental conservation strategies. The SETAC meeting is an excellent opportunity to share ideas, approaches, methodologies and preliminary results with other researchers working in the field of evolutionary toxicology.

TU 349

Association between increased resistance to copper and salinity in Daphnia longispina clonal lineages under short- and long-term multi generational exposures

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Presently, many coastal freshwater ecosystems are already exposed to chemical contamination. Depending on the period and intensity of such exposure, the selective pressure exerted by chemical contamination could have caused the disappearance of the most sensitive genotypes from exposed population, causing its' genetic erosion. Whether such eroded populations will be capable of coping with future environmental perturbations, namely those associated with the predicted climate changes such as the gradual increase of salinity in freshwater coastal lagoons, will largely depend on the association between resistance to the chemical it was exposed to and to the future environmental stressor. Accordingly, this work aimed at assessing if an increased resistance to copper is associated with an increased sensitivity to salinity; or at least if some of the genotypes most resistant to copper are among the most sensitive to salinity, both under (i) short-term exposures and under (ii) long-term generational exposures. To attain these objectives, the lethal and sublethal (growth and reproduction) sensitivity to salinity of six clonal lineages of Daphnia longispina, exhibiting different lethal resistances to copper, was assessed. Subsequently, to evaluate its acclimation to salinity, each clonal lineage was acclimated to 0.1g of NaCl for two generations and its lethal and sublethal sensitivity to salinity was re-assessed. The observed results, revealed significant associations between an increased resistance to copper and the lethal or sublethal sensitivity to salinity neither under short-term exposures nor under long-term multigenerational exposures. Also, most of the studied genotypes exhibited fitness costs in reproduction after being exposed to NaCl for more than one generation, and became acclimated to low levels of salinity.

TU 350

Metal tolerance in the earthworm Eisenia fetida: Is it a case of adaptation or mere physiological acclimation?

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In a recent study, we showed that the earthworm species Eisenia fetida, inhabiting an extremely high metal polluted compost heap on a wine farm, did not have elevated body loads of the metals but exhibited genotoxic tolerance when exposed to Cd in the laboratory (Voua Otomo and Reinecke, 2010). To unravel the mechanism behind the surprisingly low metal body burdens on one hand and genotoxic tolerance on the other hand, we investigated the estimated bioavailability of these metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total (extracted with nitric acid) metals were all below 0.05% for all four metals, suggesting low availability for uptake. The low availability of these metals could not be explained by physico-chemical properties of soil but by the phenomenon of aging of the metals. There was no difference in allozyme frequency between metal tolerant and non-metal tolerant populations of E. fetida. This suggested that the tolerance found in earlier studies could be a mere physiological adaptation.

TU 351

Potential role of biotransformation and antioxidant enzymes during maternal transfer of microcystin resistance in Daphnia

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Due to eutrophication and other factors, the frequency of cyanobacterial blooms in freshwater has increased worldwide. They are an important problem to the ecosystem, particularly affecting zooplankton populations which feed on phytoplankton. Among other toxins, some cyanobacteria produce microcystin (MC), MC-LR the most studied variant. MCs inhibit serine/threonine protein phosphatases type 1 and 2A and provoke oxidative stress. The planktonic genus Daphnia plays a central position in pelagic food

webs linking primary producers to consumers. The negative effects on Daphnia are well understood; toxic cyanobacteria affect growth, survival and reproduction. However, one possibility to ameliorate toxic effects is to biotransform MC by conjugation to glutathione via glutathione S transferase, thereby reducing toxicity and aiding excretion. This process is thought to underlie the ability to withstand MC in Daphnia and could explain the enhanced tolerance found in the offspring of Daphnia exposed to live toxic cyanobacteria.
In this study, multigeneration exposures were conducted, we exposed the parental generation to MC-LR for 1 or 7 days and determined the enzyme mediated tolerance to MC in their offspring, a) by assessing the acute effect of MC-LR on biotransformation, antioxidant and energy metabolism enzyme activities, and b) through 21 day chronic toxicity tests exposing to the same concentrations of toxin.
Seven day exposure of the parental generation to MC-LR induces higher activity of glutathione S transferase and malate dehydrogenase in the offspring and enables it to increment the catalase activity when challenged with the toxin. In offspring from the 1 day exposed parental generation those effects were less pronounced or not visible. Offspring from non-exposed and 1 day exposed mothers suffered from decreasing survival when exposed to MC-LR whereas the offspring from 7 day exposed mothers show higher survival. Higher survival of the offspring is correlated with the elevated activity of glutathione S transferase, malate dehydrogenase and catalase, suggesting maternal transfer of activation factors.
These results prove transgenerational or maternal effects provoked by a natural toxin in a very important freshwater herbivore which may explain the observed acquirement of enhanced tolerance over generations through a biochemical perspective.

TU 352
Is there a functional role of DNA methylation in the stress response?
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DNA methylation, the addition of methyl groups on the 5' position of cytosines in the DNA plays an important role in the regulation of gene expression of eukaryotic cells. Considerable research has been performed in vertebrate organisms and plants whereas DNA methylation in invertebrates has only recently received attention. In particular for Daphnia magna, a key test organism in aquatic toxicology and an important species in many aquatic ecosystems, knowledge on DNA methylation is very limited. Within our long-term aim of unravelling the potential functional role of DNA methylation in the stress response of D. magna, this study aimed at sequencing the entire cytosine methylome. For the comprehensive profiling of DNA methylation a methylated binding domain (MDB) protein based sample preparation technique was used. Since this technique is very specific and works well with fragments densily methylated, it was decided to use also a less specific technique based on DNA digestion with a novel restriction endonuclease. This endonuclease recognizes the CNNR (R=G/A) methylated sites and cleaves DNA at fixed distances generating DNA fragments easily identified on gel.

Bioinformatics analysis (i.e. mapping with the most recent D. magna genome assembly) was performed in order to generate the methylome map. Ongoing bioinformatics analyses is now focused on determining structural, functional or evolutionary relationships among the methylated sequences and to determine if methylated sequences occur in genes or pathways that may be relevant for dealing with chemical and ecological stress.

TU 353
Effects of benzo(a)pyrene and vinclozolin on freshwater snails: I) epigenetic and genetic responses of adults and II) offspring embryo toxicity
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Vinclozolin causes transgenerational effects by inducing DNA methylation changes in exposed rats. Benzo(a)pyrene is a clastogen used as a reference chemical for the in vitro mammalian cell micronucleus test. Taking into account that epigenetic and genetic profiles could be altered by these pollutants we studied molecular and cellular responses after adult exposure of freshwater snails (Physa acuta). Additionally, the embryo toxicity in the following generation (F1) after paternal exposure was evaluated. Mature snails exposed for 46 days and deposited egg masses were used for determining parental endpoints (F0) and transgenerational effects, respectively. DNA extracted from mature snails were analysed to evaluate differences in DNA methylation patterns In order to analyze methylation-sensitive sites we used PCR technique with arbitrary primers (AP-PCR) using HpaII and MspI digested samples. HpaII and MspI are isoschizomers that recognize the tetranucleotide sequence 5'-CCGG-3' but that show differential cleavage sensitivity to cytosine methylation. HpaII cannot cleave if one or both cytosines are fully methylated (in both strands), whereas MspI cleaves C5mCGG but not 5mCCGG sequences. Gel electrophoresis of PCR products and comparative analysis between HpaII and MspI patterns allowed the study of CCGG regions in the genome that are sensitive to methylation and the effect of these pollutants on these sites. Genotoxicity was measured by the micronucleus test in cells isolated from whole adults after homogenization of soft tissues. Finally, embryonic development of offspring was monitored. Eggs masses were recovered and the teratogenic effects of both non-exposed and exposed single egg capsules were measured in multiwell plates. Parental responses at cellular and molecular levels were compared with F1 responses using an ecological relevant endpoint (embryo toxicity). The aim of this work was to establish potential linkages between mechanisms of action and long-term responses at higher levels of biological organisation, which could ultimately have consequences on population dynamics. This study was supported by the Spanish research project CTM2008-03492.

TU 354
Later-life effects of early-life mitochondrial DNA damage
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I will suggest that mitochondrial DNA is a potentially important and understudied target of environmental toxicants, and one that when impacted is likely to have long-term effects. Mitochondrial DNA (mtDNA) is more sensitive than nuclear DNA to many common genotoxins, and lacks some repair pathways that are present in the nucleus. In particular, helix-distorting adducts formed after exposure to environmentally important genotoxins such as ultraviolet C (UVC) radiation and some polycyclic aromatic hydrocarbons and mycotoxins are repaired in the nuclear genome by nucleotide excision repair. However, nucleotide excision repair proteins are not present in the mitochondria, so that the fate of such DNA damage in the mitochondrial genome is unknown. The effects of such persistent damage are also unknown; we hypothesized that the effects of such damage would be particularly important after early life stage exposure since mtDNA copy number is lowest at that time. Using the nematode model Caenorhabditis elegans, we found that UVC-induced photodimers result in lower levels of mtDNA-encoded mRNAs, decreased ATP levels, decreased oxygen consumption, larval developmental arrest, and neurodegeneration. Furthermore, UVC-induced mtDNA damage is slowly removed in a process dependent at least in part on mitochondrial fusion, fission and autophagy. Mutations in mitochondrial fusion and autophagy genes exacerbate the larval arrest, suggesting a potent gene-environment interaction in which the effects of mtDNA damage caused by environmental agents are exacerbated by decreased mitochondrial fusion and autophagy.

TU 355
Is trans-generational PCB transfer a key process for parental detoxification and adaptation in amphipods?
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To date, reproduction success and reproductive disorders have been studied in several marine organisms like fish and amphipods to address the question of the impact of contaminant exposure on wild populations. This study is focussed on the characterisation of the progress of toxic effects from subcellular damage at the individual level of the parental generation to deterioration of the offspring in the amphipod species Monopoireia affinis. Monoporeia affinis have been sampled at differently polluted and reference areas of the Bothnian Bay. Reproduction success in amphipods includes various variables as e.g. fecundity, sex ratio, sexual development, embryo aberrations in terms of malformed and membrane damaged embryos, undifferentiated and dead eggs and broods, and stage of embryo development. In addition, amphipods were sampled for the in situ assessment of lysosomal membrane stability (LMS) as an indicator of toxic damage in the parental generation.These studies were combined with chemical analysis (PCBs, PAHs, and metals) of sediment and organisms to gain information about potential cause-effect relationships. The integrated analysis of the data showed that the nature of assessed correlations between contaminant concentrations in sediment, contaminant concentration in amphipods, parental damage, and reproduction success differ between the contaminants. For PCBs, high sediment concentrations correspond with low concentrations in amphipods and vice versa.The concentration in amphipods is negatively correlated with lysosomal membrane stability and embryo malformation.High membrane stability and low PCB concentrations in the parental generation corresponds with high fecundity and vice versa. Based on these results, a conceptual model on the tranfer of PCB during embryogenesis has been developed. It is hypothesized that PCBs are taken up by the amphipods according to the respective bioavailabilty. During embryogenesis, PCBs are transported to the gonads together with nutrients and yolk. Thus, PCB concentrations in the parental generations are low and membrane stability and fecundity high. The PCBs act toxic on the developing embryo instead. In case of high PCB concentration in the parental generation, fecundity is reduced and embryo malformations low. These results indicate an adaptation to high PCB concentrations possibly mediated by a limited transport of PCBs during embryogenesis.

TU 356
The enzymatic functional stability of zinc-tolerant microbial communities: a new approach to detect terrestrial ecotoxicity of metals
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The soil microbial community exposed to metals over a long period can acclimate or adapt their biogeochemical functions and become metal-tolerant. This tolerance could increase the vulnerability of the microbial community to additional soil disturbances and then decrease the soil microbial functional stability. The functional stability is defined as the ecosystem capacity to recover its biogeochemical functions after a disturbance. This concept is more and more studied because this approach could detect ecotoxic effect which would have not been found out by common microbial bioindicators. A method which assesses soil health by analyzing the functional stability of soil enzymes has been set during the last years and has developed a stability indicator named the Relative Soil Stability Index (RSSI). These indexes were calculated to assess the enzymatic functional stability of 9 zinc-contaminated field soils after a heat disturbance (desiccation at 60°C for 24h). Four enzymes were analyzed: arylsulfatase, acid phosphate, protease and urease. The RSSI of arylsulfatase and protease were linearly correlated to the labile zinc concentration in the soils (arylsulfatase : $R2= 0,65$ for $n=7$ and protease : $R2=-0,62$ for $n=6$, $\alpha < 0,05$), while the enzymatic activity of these enzymes was not correlated to the labile metal fraction. These results suggest that the sulfur and nitrogen cycles would be unstable in zinc contaminated field soils. This potential ecotoxic effect would not have been detected if only the enzymatic activity has been analyzed.

TU 358

Does genetic adaptation matters? An hypothesis tested using life-history consequences of adaptation and acclimatization to copper of Daphnia longispina

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The present study was conducted to test the hypothesis that genetically adapted clones of Daphnia longispina showed a higher fitness performance under copper exposure than acclimated clones. Genetically tolerant and sensitive clones were selected from a reference and a historically exposed populations to an acid mine drainage from an abandoned pyrite mine. Four sensitive and resistant clones from reference and impacted populations were exposed to a range of copper exposure levels and their life history responses in terms of survival, reproduction and population growth rates compared. In another experiment the most sensitive clone was exposed during four generations to the same range of copper concentrations and its life-history performance compared. The genetic differences between the resistant and sensitive clones were that in the polluted environment individuals of the resistant clone survived 50 % better, reproduced three days earlier, produced three more offspring per day and had population growth rates 45 % greater. In the unpolluted environment, however, individuals from the resistant clone had the lowest reproduction rates. After four generations acclimated individuals from the sensitive clone reproduce earlier, had greater clutches at first reproduction and higher population growth rates than none acclimated ones but apparently there were no fitness costs. Therefore, our results showed that life history changes due to adaptation and acclimatization were comparable under the polluted environment but not under a clean environment. This results support the view that genetic adaptation is ecologically costly but acclimatization not.

TU 359

Evaluating effects of pollution on Caenorhabditis elegans' population dynamic through a bio-energetic approach

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The assessment of toxic effects at biologically and ecologically relevant scales is an important issue in ecosystem protection. Mathematical models exist to predict effects of pollutant on population dynamics from individually data. Nevertheless there are only a few datasets and models that account for adaptive phenomena which may appear in a stressed population. The selection pressure exerted by a pollutant is known to amplify the phenomenon of natural selection. It is thus essential to understand and quantify the adaptive dynamics governing populations under stress in order to assess ecological risk.
Regarding this background, we adapted a bioenergetic model to study adaptive phenomena in Caenorhabditis elegans population dynamic exposed to a heavy radiotoxic metal (uranium). The Dynamic Energy Budget (DEB) (Kooijman, 2010) bioenergetic approach highlights the distribution of energy fluxes between processes such as growth, reproduction, maturation and maintenance. It is a relevant basis to understand and model the links between assimilation disruptions, growth and reproduction fluctuations in organisms exposed to anthropogenic stress (e.g. pollutant, global change) and to assess potential consequences on population over many generations.
We therefore studied the responses of C. elegans exposed to six experimental concentration of uranium over several generations. The individual traits followed were growth curve, egg laying curve, survival until end of egg laying. We showed that uranium impacted C. elegans growth curve and egg laying over several generations, with, consequently, adverse effects on the population dynamic and variations on DEB parameters. Nevertheless, results also tend to show an evolutionary response throughout the generations.

ET11P - Marine environmental chemistry and ecotoxicology

TU 361

Biotechnological potential of impacted scenarios for the restoration of TBT contaminated environments

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Tributyltin (TBT) is an organotin compound commonly used as an antifouling agent in marine paint formulations. Due to its wide industrial application and its consequent discharge into the environment, TBT pollution is recognized as major environmental problem at a global scale, being recently considered to be the most toxic substance ever introduced into the marine environment. Microorganisms from historically contaminated sites are able to tolerate pollutants and even degrade them, which may be a key factor in the restoration of contaminated environments. Nevertheless, byproducts resulting from the degradation process might be more or less toxic than the parent compound to ecological relevant species. The determination of the substance presence by analytical chemistry, although essential, may not present ecological relevance, as it might not be related to its ecotoxicity.
In this study, TBT-resistant bacteria collected from 7 Portuguese ports (Póvoa de Varzim, Leixões, Aveiro, Figueira da Foz, Peniche, Setúbal and Sines) were isolated in increasing concentrations of the toxicant (0.1, 1, and 3mM of TBT) and those growing at the highest concentration were characterized by genomic fingerprinting (REP-PCR) and tested as potential bioremediation tool in laboratory contaminated media.
The percentage of TBT-resistant isolates varied between 0.08% (Setúbal harbor) and 7.67% (Peniche). REP-PCR analysis revealed a total 111 distinct genetic profiles, being Peniche the location with lower variability while Figueira da Foz had the highest variability. Selected isolates were used to bioremediate waters contaminated waters, and their potential as bioremediation tools was assessed through ecotoxicological testing with the gastropod Gibbula umbilicalis. Ecotoxicological testing suggested that some TBT-resistant bacteria are able to reduce the toxicity of TBT contaminated waters. Ecotovxicological testing also revealed that the median lethal concentration (LC50) of TBT in Gibbula umbilicalis at 48 and 96 hours was 61.45 µg L-1,and 15.69 µg L-1, respectively.
This study contributed to the understanding of TBT resistance, however more intensive and focused research in the area of TBT bioremediation mediated by marine bacteria is still needed, particularly on the mechanisms behind TBT resistance and on the identification of pathways and genes responsible for TBT degradation.

TU 362

Bacterial diversity on Portuguese ports: TBT degrading bacteria

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Tributyltin (TBT) is an organotin commonly used as an antifouling agent in marine paint formulations. Due to its wide industrial application and discharge into the environment, and severe effects caused by it (e.g. imposex), TBT pollution is recognized as major environmental problem at a global scale.
Microorganisms from historically contaminated sites are due to tolerate pollutants and even degrade them, which can be key factor in the restoration of contaminated environments.
We have isolated 157 TBT-resistant isolates from Portuguese ports and harbours. Bacteria resistant to 3mM TBT were characterised by rep-PCR. Relationships between the PCR-generated patterns were examined by cluster analysis. Among the 157 isolates examined, 111 different banding patterns were identified. We have characterized these isolates in what concerns growth rate in the presence of increasing concentrations of TBT and later identified them by 16S rRNA gene sequencing. Bacterial diversity was also assessed by DGGE.
We were able to detect TBT-resistant bacteria from all the sampling sites. It is possible to observe that with increasing concentrations of TBT, the concentration of isolates decreased and consequently the percentage of resistant bacteria compared to the control was lower. At 0.1mM, Póvoa de Varzim had the highest percentage of resistants' (45.5%) while Setúbal had the lowest (2.56%). Regarding 1mM and 3mM concentrations, Peniche had the highest percentage rate (8.2% and 7.7% respectively) as well the highest number of colony forming units per milliliter values (410 ± 26 cfu mL-1 and 383 ± 91 cfu mL-1 respectively). On the other hand Aveiro (0.26 ± 0.09%) and Setúbal (0.08 ± 0.03%) had the lowest percentage of resistants' for 1mM and 3mM, respectively. Aveiro had the lowest isolates concentration in the control, while Peniche had the highest. Statistically significant differences were observed for all locations between selective mediums and control groups.
This study contributed to the understanding of TBT resistance, namely to get insides into the constitution of TBT-resistant communities in port and harbour waters.

TU 363

Are TBT sources migrating from harbors to marinas in Latin America?

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Tributyltin (TBT) is a very toxic compound that was used as antifouling paints biocide during four decades. Due its environmental toxicity the International Maritime Organization banned TBT based antifouling paints in September 2008. Thereafter, TBT environmental concentrations as well as imposex levels (the most widely used TBT biomarker) declined in several coastal areas worldwide. However, in Latin America some recent observations have shown high TBT and imposex levels in areas close to marinas, despite the general reducing pattern in many areas. The present study reported imposex parameters (imposex %, VDSI and RPLI) in gastropods from Venezuela and Brazil in harbor areas and marinas. In Venezuela, samples with 30 adults of Purpura patula were collected in 23 sites (2 located inside marinas). In Brazil, the sampling

collection has been performed for some years at Angra dos Reis (10 out of 33 stations were close to marinas) and Arraial do Cabo (2 out of 10 stations were close to marinas) in Rio de Janeiro state. These studies used 30 adults of Stramonita haemastoma per site (whenever found). The results for Venezuela showed that the VDSI ranged from 0.22 to 1.86 in areas under the influence of harbors, whereas VDSI values were 3.77 and 4.56 in the marinas. Similarly, RPLI ranged from 0.07 to 5.58 in the harbor areas and were 49.36 and 54.56 to the marinas. In this case, the main source of organotins pollution is clearly the marinas. In the Angra dos Reis study, imposex was detected in 100% of females close to the marinas, and in 5 out of 10 stations the animals previously inhabiting the rocky shores were no longer found. In Arraial do Cabo, two stations nearby a single marina showed an imposex incidence of 4% and 0 in 2001, increasing to 83% and 47% in 2008. In this region, another marina located nearby a small harbor showed imposex incidence of 100% in three stations in 2001, whereas no gastropods were found during 2008 sampling. Thus, although the international restrictions on TBT use are apparently reducing the impacts from harbors, these three independent studies at Venezuela and Brazil (Angra dos Reis and Arraial do Cabo) clearly pointed out that marinas are becoming an important source of TBT pollution. Based on that, a similar pattern is expected to occur in other Latin America coastal areas.

TU 364

Imposex and organotin (OT) levels in Nucella lapillus along the Portuguese coast: a re-survey in 2011

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The use of tributyltin-based antifouling paints (TBT-based AFP) is globally forbidden since the 17th September 2008 by the 'International Convention on the Control of Harmful Antifouling Systems on Ships' (AFS Convention). It is therefore important to ensure the continuous monitoring of organotins (OT) in the environment and the recovery of marine ecosystems to evaluate the effectiveness of legislation in reducing TBT pollution. The current work assessed imposex and OT tissue contamination in *Nucella lapillus* at sixteen sites along the Portuguese coast in 2011. The vas deferens sequence index (VDSI), the female penis length index (FPLI), the relative penis size index (RPSI), the percentage of imposex affected females (%I) and the percentage of sterile females (%S) were determined to assess imposex levels. Additionally, monobutyltin (MBT), dibutyltin (DBT), TBT, diphenyltin (DPT), triphenyltin (TPT), monoctyltin (MOT) and dioctyltin (DOT) were quantified in the soft tissues of whole females by GC-MS. The OSPAR Ecological Quality Objective (EcoQO) set for this species (VDSI < 2) was achieved in 94% of the sampled sites. All surveyed populations presented VDSI values that fall into OSPAR class B (0,3 < VDSI < 2) with one single exception in the South western coast - Zambujeira do Mar (site 15) - also being the only site where sterile females were still recorded. OT concentrations in *N. lapillus* soft tissues varied between <0.2ng Sn/g and 24ng Sn/g dry weight (dw) for MBT, 2.0 and 26ng Sn/g dw for DBT, 2.3 and 30 ng Sn/g dw for TBT and <0.2 ng Sn/g and 1.4 ng Sn/g dw for TPT. DPT, MOT and DOT concentrations were all below the detection limit. TBT recent inputs were estimated to occur at four sites by the butyltin degradation index (DBI = ([MBT]+ [DBT])/ [TBT]). The temporal trend of *N. lapillus* imposex was analysed for the period 2000-2011 in order to assess the evolution of TBT pollution levels in the Portuguese coast.

TU 365

TBT from anti-fouling paints in the environment - an overview of the situation today

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The Baltic Sea is a sensitive brackish water environment where most organisms live at the edge of their distribution area. Pollutants thus mean an extra stress to these organisms. Traditionally anti-fouling paints are based on leakage of biocides, which prevents the attachments of fouling organisms on underwater structures such as a boat hull. The draw-back is that these biocides are persistent and that they also affect non-target organisms. The environmental authorities have with time implemented more strict regulations, especially for the paints that may be used on the east coast of Sweden. The use of tin-organic substances were prohibited in 1989 for all boats less than 25 m and no paints based on copper leakage were approved from 2001 and up today no copper paints have been on the market for use on the east coast. In spite of these restrictions much toxic substances originating from anti-fouling paints are found in the sediment in both natural harbours and in marinas along both the west coast and the east coast of Sweden. Similarly high concentrations are found in harbours for ships. Our investigations show that the highest concentrations are found in boat yards > uptake areas > marinas > natural harbours. 100 µg TBT/kg DW is considered high in many countries and the highest concentrations in boat yards reached 50 000 µg TBT/kg DW. The reason is under laying paint layer being scraped off.

TU 366

Comparing anadara trapezia exposure, dose and response to metal contaminated estuarine sediments using laboratory and field exposures and resident molluscs

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Establishing relationships between metal exposure, internal dose and associated biological effects for organisms is necessary to understand the fate and effects of metals in the environment.
The accumulation and sequestration of biologically available metals by aquatic organisms, particularly bivalve molluscs, has led to their use as biomonitors of metal contamination, as it is this portion which is of interest in pollution effects assessments. Biomarker measurements can provide evidence that organisms have been exposed to contaminants at levels that exceed their detoxification and repair capacity, thus establishing the link between contaminant exposure and ecologically relevant effects. Laboratory exposures, in which physiochemical factors can be controlled, using previously unexposed organisms are routinely used in the development of toxicological effects tests. Metal accumulation is influenced by metal bioavailability via water, sediment and dietary pathways. These pathways may be influenced by mode of exposure and the history of previous exposure which in turn may affect biological responses.
An approach for establishing the relationships between tissue metal dose and biological response of exposure to copper, zinc, cadmium, lead, and selenium contaminated sediment from Lake Macquarie, NSW, Australia, was evaluated for the sediment dwelling bivalve mollusc Anadara trapezia. A comparison of tissue metal accumulation and effects was made between resident organisms, and previously unexposed organisms in laboratory and in situ caged exposures. Organism dose was measured by total metal tissue burden. Subcellular fractionation of whole tissue was used to determine what fraction of the total accumulated tissue metal was in a metabolically available form. Measurements of oxidative stress (total antioxidant scavenging capacity of cells and lipid peroxidation) were used as effects biomarkers of metal exposure. Lysosomal membrane stability was used as a measure of cellular integrity. By identifying relationships between exposure and effects at various levels of biological organisation, using a variety of exposure approaches, a better understanding of the mechanisms of organism stress responses to metals in ecological systems was gained and the predictive capability of ecological risk assessment improved.

TU 367

Imposex occurrence and the genetic changes in Veined Rapa Whelk (Rapana Venosa) from Bohai Bay, China

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In order to assess the present status of organotins (OTs) pollution and the potential ecological risk, biological effects in biota and the contents of OTs in biota and sediments were investigated in Bohai bay, north of China. Imposex occurrence was found in veined rapa whelk (RaPana venosa) from three sites in Bohai bay (HH, NPH and DST), ranging from 0% to 12.45% in 2010 and from 0% to 12.50% in 2011, respectively. The chemical analysis results showed that TBT and TPT were the main OTs in tissues and sediments, which can account for the occurrence of imposex. Furthermore, the genomic DNA that was amplified with 8 pairs of fluorescence-labeled primer showed varied genetic distances and Nei's genetic identity among the veined rapa whelk from the 3 sites. The present study demonstrated that the biota was contaminated by OTs in the Bohai bay, and this imposed a potential risk to the veined rapa whelk.

TU 368

Clam valve rhythm-based online biomonitoring system to detect waterborne arsenic

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Arsenic (As) usually represents high level in groundwater at southwestern and northeaster coastal areas of Taiwan. The freshwater clam has a high market value and commercially important to Taiwan's aquaculture. Previous studies indicate that biomonitoring is the scientific technique for detecting environmental pollution situations for presenting the harmful of toxic chemical in water. The purpose of this study was to synthesize water chemistry-based bioavailability and valve daily rhythm in Corbicula fluminea to design a biomonitoring system for detecting waterborne As. We integrated valve daily rhythm dynamic pattern and water chemistry-based Hill dose-response model to build in a valvometry technique programmatic mechanism, offering a rapid and cost-effective dynamic detection system. We validated the simulated dissolved As concentrations based on valve daily rhythm behavior with published experiment data. The results indicated that the As concentration detection threshold of biomonitoring system should be less than 1 mg L^{-1} and the detection times are associated with the exposure concentrations. This study presents a *C. fluminea*-based biomonitoring system that can particularly provide the real time transmitted information on the waterborne As activity. This parsimonious *C. fluminea* valve rhythm behavior-based real-time biomonitoring system presents a valuable effort to promote the automated biomonitoring and offers early warnings on the potential ecotoxicological risks in regions with elevated As exposure.

TU 369

A comparative in situ study on ecotoxicological effects of pharmaceuticals in Ireland, using marine mussels (Mytilus spp.)

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In recent decades the amount of pharmaceuticals used and released has constantly increased. One of the main sources of this pollution is through the release of wastewater

effluent, both treated and untreated, into the aquatic environment. These novel contaminants can be found now through the developed world, including Ireland.
In our field study, we collected blue mussels (*Mytilus* spp.) from a pristine site in the west of Ireland (Lettermullan, Co. Galway). They were caged and exposed at three sites around Ireland for three months in 2010. Cages at site one were based above the wastewater diffuser pipe of Mutton Island wastewater treatment plant (WWTP), a secondary treatment facility serving Galway city with a population equivalent (p.e.) of 73,000. This WWTP treats both municipal and industrial waste. The second location was at the North Bank Lighthouse in Dublin Bay. This lighthouse is based 700m downstream of the Ringsend WWTP, which is a tertiary treatment facility. Wastewater from homes, industries, commercial premises as well as rainfall water is treated in Ringsend, which serves a p.e. of 1,7 million. Omey Island, Co. Galway was the control site for this study. It is a remote island on the Galway coast, with little anthropogenic input.
The protein expression signature and other general stress responses were determined by measuring changes in a suite of biomarkers comprising glutathione S-transferase, lipid peroxidation, DNA damage and vitellin-like proteins.
Our results contribute to the understanding of whether chronic exposure of pharmaceuticals causes a real threat to aquatic organisms, their habitat and, ultimately, to humans health.

TU 370
Chronic exposure of young spat of the Pacific oyster (Crassostrea gigas) to zinc
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The marine coastal environment is constantly submitted to many sources of releases from human origins, resulting in low concentrations of contaminants and most coastal marine species are exposed to these low doses during their entire life. Because biological mechanisms to respond to acute and chronic exposure may be different, the concentration sensitivity is also different. It is thus a challenge that stake holders in charge with the environment protection regulation can lean on robust scientific basis addressing the consequences of chronic exposure of species to low doses of pollutants. For long life-span species, investigating chronic exposure means long term experiments with many potential technical biases. A relevant strategy is to use early stages of development. Even if the exposure time is limited compared to the life-span of the organism, if exposure starts at the very beginning, it covers the whole lifetime up to the end of the experiment, which is relevant in terms of chronicity. In addition, early stages of development are often more sensitive to pollution than adults, so using them for ecotoxicology obviously makes sense. Zinc is an essential trace metal in living organisms but it becomes toxic at high concentration. In the coastal marine environment, its concentration is influenced by many human activities, among which the use of antifouling paint and sacrificial anodes. Our study addressed the effects of zinc on Pacific oyster (*Crassostrea gigas*) spat. Young oysters were exposed to a range of zinc concentrations, added to seawater as $ZnCl_2$ up to 2 $mg.l^{-1}$, as of 1 day post-metamorphosis and for 10 weeks. They were fed with micro algae at the same cells concentrations. Zinc was measured in seawater and in oysters throughout the experiment. Survival and growth were monitored on large sample populations to deal with individual variability. A dose-response curve analysis allowed deriving the EC_{50} for growth. In parallel, target genes analysis included heat shock proteins, metallothionein (MT), detoxication machinery, regulation of oxidative stress and cell cycle. Gene transcriptional expression was quantified by RT real time PCR. Significant change was only detected for MT after 1 week exposure to the highest zinc concentration. However growth was affected as early as a few days of exposure to relatively low dose and turned out to be an earlier and more sensitive marker of the effect of zinc on oyster spat.

TU 371
Assessment of sacrificial anode impact by zinc accumulation in oyster Crassostrea gigas: comparison between a long- and short-term laboratory tests
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In marine environments, sacrificial anodes made of zinc are currently used to mitigate marine corrosion as part of cathodic protection systems of immerged metallic structures. Zinc is an essential metal for all living organisms that can be potentially toxic when present in excess. The aim of this study was to assess the bioaccumulation and the effects of zinc released using an electrochemical device providing controlled zinc concentrations from sacrificial anode degradation to mimic the in situ conditions. The work was carried out on oyster Crassostrea gigas by performing two in vivo tests. The first test was conducted over a period of 10 weeks at a concentration of 0.53 mg Zn L-1 to simulate long-term exposure, and a second one lasted for 168-hours at a concentration of 10.2 mg Zn L-1 to reproduce short-term exposure. During exposures, zinc concentrations were measured in oyster gills, digestive gland and soft tissues and, endpoints such as mortality, immune parameters and mRNA expression of genes associated with oxidative stress and cell detoxification were analysed.
At the end of the 168-hour exposure experiment, 81.8% mortality rate was recorded, and the bioconcentration factor (BCF) exhibited values up to 405 in the digestive gland. In contrast, the results obtained though chronic exposure showed a higher BCF (13,397), whereas no mortality was detected. Moreover, zinc was able to modulate immune system activities. Indeed, all of the immune system biomarkers studied, except the number of circulating haemocytes which decreased in both experiments, were stimulated by a moderate level of zinc and inhibited by a high level. Our exposure conditions did not induce SOD or MXR mRNA expression. However, a dose- and time-dependent increase of MT mRNA correlated with Zn accumulation in gills and digestive gland of exposed oysters was observed. These results demonstrate the great capacity of Crassostrea gigas to accumulate zinc released from anode, especially when low concentrations are released, as in the case of anode dissolution used as cathodic protection. In addition oyster physiological endpoints (e.g. immune parameters, stress biomarkers) are sensitive to acute Zn toxicity but are only moderately affected by a mild Zn concentration. This study confirmed the necessity to monitor this zinc contamination source in marine environments.

TU 372
Effect of herbicides on embryo-larval development and metamorphosis in the Pacific oyster, Crassostrea gigas
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The summer mortality syndrome which sporadically threatens oyster farming appears to be due to a combination of several intrinsic and extrinsic factors including physiological stress related to reproduction and xenobiotic stress. In this context, terrestrial inputs including pesticides could be involved in oyster mortality events, not as a single causative agent but as additional stressors. The study was carried out in the frame of the European Projet Chronexpo. The aim of this project was to evaluate the impact of different contaminants (including pesticides) used in the region surrounding the English Channel on the early stages of Crassostrea gigas. Experiments were conducted on glyphosate, its main metabolite (AMPA), two commercial formulations (Roundup Express' and Roundup Allées et Terrasses') and also adjuvant (POEA: Polyethoxylated tallowamine) alone. Mecoprop, Mecoprop-P and their main metabolite (2-methyl-4-chloro phenol; 2M4C) were also tested.
The effects of these substances and copper sulphate (used as a positive control) were studied in D-hinged larvae in order to assess their potential embryotoxicity by considering both the rate of abnormal larvae (countings) and the kind of abnormalities (multi-variated analyses). The effects were also researched in pediveliger larvae ready to metamorphosis (calculations of metamorphosis rates). For metamorphosis test, we optimized Coon & Bonar's protocol because of the difficulty to count moving larvae. For each endpoint (embryo-larval development and metamorphosis rates), results of 4 experiments allowed us to determine the EC50 values.
As regards embryotoxicity, the 2 kinds of Roundup appeared about 10-fold more toxic than glyphosate and AMPA and the toxicity of 2M4C reached 3-5-fold those of active substances. In the metamorphosis assay, a similar result was recorded and, glyphosate, AMPA and mecoprop appeared little toxic (EC50 >100 mgL-1). Finally, the comparison between the 2 larval stages generally showed a greater sensitivity of D larvae (embryotoxicity) compared to pediveliger larvae. For most of the molecules, results showed that embryotoxicity assay is more sensitive than the classic standardized acute toxicity Daphnia magna test. This work is the first part of a study which will be continued by long time exposures and will allow us to provide guidance on pesticide regulation in the frame of the European Directive "Marine Strategy"(2008/56/CE).

TU 373
Ecotoxicity of nitramines, important transformation products of amines used in carbon capture
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Due to the imminent use of amines in the CO2 capturing process from gas power stations in Norway, it is anticipated that the inputs of amines and transformation products into the environment will increase. The two main groups of transformation products with the most potential to cause environmental harm have been identified as nitrosamines and nitramines, both of which are considered to be carcinogenic. Recent theoretical modelling as well as laboratory experiments have found nitramine compounds, 2-(nitroamine) ethanol (CAS: 74386-82-6) and dimethylnitramine (CAS: 4164-28-7) to be present. However, despite the likelihood of these compounds increasing in the environment, no environmental toxicity data for these compounds currently exists. Therefore, the aim of this work was to provide an environmental risk assessment for the selected nitramine compounds taking into account the key trophic groups within freshwater, marine and terrestrial environments. The toxicity assessment was made using a suite of standardised bioassays for the measure of acute and chronic toxicity, which found the EC/LC50 concentration of the selected nitramine concentrations to be in the low to mid mg/L range for all aquatic toxicity tests. In addition, the sub-lethal effects of these compounds was also investigated, using in a 3 tiered approach to assess the potential mutagenic and carcinogenic effects of these compounds. Overall, data to develop the environmental risk assessment for these future environmental pollutants will be presented.

TU 374
Effects of arsenic on physiological parameters of mussels (Mytilus edulis) from the Scheldt Estuary (Belgium)
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Mussels are marine organisms widely used as environmental biomonitors, due to the ability to concentrate pollutants in their tissues with minimal metabolic transformations.

Arsenic is widespread in the marine environment and occurs in several chemical forms, principally as arsenate (As V), and to a lesser extent as arsenite (As III) a more toxic form of arsenic. Arsenic introduced into the environment often contaminates aquatic ecosystems, where the residence time has been estimated at about 50 years. This study aimed to determine whether exposure to specific heavy metal arsenic has an effect on the physiology of mussels. The effect of arsenic in mussels were analyzed in an experiment with 150 mussels divided in 7 different groups and exposed to 6 different concentrations of As(III) and one control for 10 days, during this period several physiological parameters were analyzed (Scope for Growth, energy reserves, body condition index, Mts and arsenic accumulated by the mussels). Arsenite does not seem to affect the physiological parameters of Mytilus edulis, but the organisms can accumulate As (III) proportionally to the concentrations that it is exposed, especially at high concentrations. Several authors were not able to find this relation, but this is due to the low concentrations used in their experiments. The present study has shown that the arsenic accumulated in the body of mussels did not have adverse effects to the mussels, at least in a short period of time and for those physiological parameters measured, but after 10 days of exposition some parameters (lipids and SFG) have positive correlation with arsenic in sea water, suggesting an effect of arsenite. Long-term experiments should be performed to really understand these effects.

TU 375

Changes in the biomarker responses of the razor clam Solen marginatus as an indicator of varying contamination exposure in two Portuguese coastal lagoons: a case study

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Coastal lagoons are highly productive marine environments that provide a wide range of ecosystem services. Nevertheless, these habitats are particularly vulnerable to direct and indirect sources of pollution, which leads to environmental degradation and ecosystem impairment.
The aim of this study was to monitor the health status of two natural populations of *Solen marginatus* from Ria de Aveiro and Ria Formosa, two Portuguese coastal lagoons located in Aveiro and Algarve, respectively. In order to achieve this, individuals from both places were sampled, in two successive years, and an additional group of individuals was sampled from a reference site (São Jacinto, Aveiro). For each sampled population and sampling time, lipid peroxidation and the activity of glutatione-S-transferase, catalase and cholinesterase were determined. In addition, total mercury burden in whole organisms was also monitored as mercury is one of the major contaminants that can be found in these costal lagoons. Significant differences were observed between the two lagoons regarding the mercury burdens accumulated and the enzymatic activities measured, suggesting that the two populations are exposed to different environmental quality and contamination levels.

TU 376

Marine ecosystem health assessment through the Integrative Biological Index (IBI) in mussels from Portugal NW and Basque coasts

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In order to assess the biological effects of pollution, mussels *Mytilus galloprovincialis*, were seasonally sampled in seven localities subjected to different levels of pollution along the North Iberian Peninsula Coast (NIPC) over one year (April, June, October 2010 and in February 2011); 4 in the NW Coast of Portugal (Vila Chã, São Bartolomeu do Mar, Viana do Castelo and Carreço) and 3 in the Basque Coast (Arriluze, Gorliz, Mundaka). In each locality, a battery of biochemical and cell and tissue-level biomarkers were applied: acetylcholinesterase (AChE) and glutathione S-transferases (GST) enzymatic activity, levels of lipid peroxidation (LPO), lysosomal enlargement (lysosomal volume density, Vv), lysosomal membrane stability (labilisation period, LP), cell type replacement (volume density of basophilic cells, VvBAS) in digestive gland epithelium, and changes in the morphology of digestive alveoli (mean luminal radius to mean epithelial thickness MLR/MET). These biomarkers were integrated in the *Integrative Biomarker Index* (IBI), which was developed within the framework of the Prestige oil spill (POS). The IBI index revealed that mussels' health was affected in chronically polluted localities. Star plots accompanying IBI provided complementary information concerning mechanisms of biological response to environmental insult. The NIPC is a high risk area for marine spills due to intense marine traffic. This is an important integrative field study to understand the present environment health status and to determine the levels of general stress on core localities which provide reliable assessment of future impacts of pollutant. This study was supported by the Government of the Basque Country through a post-doc grant to L. Garmendia (ref. BFI09.244) and K-EGOKITZEN project and by the Portuguese Foundation for the Science and Technology and FEDER funds through the project RAMOCS (ERA-AMPERA/0001/2007; EU AMPERA ERA-NET, ERAC-CT2005-016165).

TU 377

Metal exposure and associated effects in mussels: an integrated approach - hyphenated analytical techniques and biomarkers

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Aim of the study was the assessment of metal exposure in coastal areas under indirect or direct influence of maritime activities and nautical tourism (port and marinas) using integrated biological-chemical analytical approach and mussels (*Mytilus galloprovincialis*) as bioindicator species.
Next to the natural sources, a large number of metals are present in the environment as a result of human activities. Since the ban of organo-tin compounds, Cu is the main component of anti-fouling paints, with the result that boat traffic is a major anthropogenic source of Cu in the coastal aquatic environment. Besides, zinc oxide is generally used in combination with Cu(I) as a booster. As a result of the leaching of chemicals from the painted surface, concentrations of metals are significantly elevated in semi-enclosed marine systems, such as harbours, marinas and estuaries, where the transport, berthing or docking of vessels is extensive.
In this study we applied new methodological approach - investigation of metal distribution in the cytosolic fractions of proteins separated by size exclusion chromatography (SEC-HPLC) and detected by inductively coupled plasma mass spectrometry (ICP-MS). Additionally, the cellular energy allocation (CEA) methodology was applied as a general biomarker of physiological status of mussels. Supplementary, a non-target bioanalytical approach utilizing 2D gel-electrophoresis will give hints for further potential protein biomarker. Thus, this study represents a contemporary and new approach in quantification and assessment of the environmental risk caused by metal exposure, as well as the potential toxic effect of metals on mussels.
The study was performed on indigenous mussel populations sampled in the middle part of the eastern coast of the Adriatic Sea at the locations of two ports, two marinas and a reference (unpolluted) site. The results obtained from the sampling in March 2011 will be presented.
This study was performed within the scope of the DAAD project (Project-ID 50752021 Deutscher Akademischer Austausch Dienst) during which scientific collaboration between the research groups from Croatia and Germany was established.

TU 378

An improved on-line clam-based behavioral monitoring system allows an approach to estimate dose-response profiles of clams exposed to waterborne copper

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The purpose of this study was to develop an improved on-line clam-based behavioral monitoring system. The system included a valvometry apparatus and three kinds of valve closure analytic programs that can offer a real-time and cost-effective method to construct an approach for estimating dose-response profiles of clams (*Corbicula fluminea*) exposed to waterborne copper (Cu). The valvometric technique can reduce the environmental stress of observed bivalves to promote a measuring precision in the spontaneous status of recording daily valve movements. The clam-based on-line behavioral response monitoring system was used to respectively monitor the valve closure responses of 16 *C. fluminea* exposed to unpolluted environment and various Cu concentrations. In this study, a probabilistic-based approach describing the valve behavioral response of *C. fluminea* exposed to unpolluted environment and Cu was developed. The magnitudes of shell gape of 20 and 50% were respectively adopted as the determining thresholds of the valve closing (VC) and siphon extension (SE) status to digitalize the valve movements in bivalves. The observed data of valve closure response was analyzed to construct the time-varying dose-response profiles (*RVC* and *RSW*) based on an empirical three-parameter Hill model. It also allowed the estimation of the integration time-specific $EC50^{VC}$ and $EC50^{SW}$ values as a bioassay approach. The daily valve opening and closing rhythm were characterized by a three-parameter lognormal function. The time-specific $EC50^{VC}$s and $EC50^{SW}$s were accordingly estimated to be 70.56, 48.78, 35.85, 19.8, 6.54, 4.97 and 73.2, 43.69, 19.52, 8.77, 6.58, 5.61μg L-1, respectively, at integration times of 10, 15, 30, 60, 120 and 300 minutes. The results revealed that the *RSW*-based bivalve behavioral observation has a better sensitive response for detecting a lower waterborne Cu concentration than that of *RVC* within one hour. The results also demonstrated that the response sensitivity of clams exposed to Cu depended on the initial valve opening and closing state of *C. fluminea* after Cu occasional addition, indicating that clams in response to different Cu exposure concentrations resulted in various valve closing rates. In the future, an estimation of dose-response profiles of clams exposed to waterborne contaminant should take into account the related response characteristics of valve activities to promote the predictive capabilities of this bioassay approach.

TU 379

Glutathione S-Transferase, glutathione peroxidase and acetylcholinesterase activities in mussels transplanted to harbour areas

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As part of an integrative monitoring campaign involving water and sediment chemistry, in situ bioassays, and mussel bioaccumulation and biomarkers, Mytilus galloprovincialis mussels of standard size were transplanted from a clean location to five sites in two important harbours from the Atlantic coast of Spain (Vigo and Pasaia) at the 2008 year. After a 30-day field exposure, the enzymatic activity levels of glutathione S-transferase (GST), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) were quantified in the gills of transplanted mussels (n=12). GST activity is induced by organic pollutants, GPx activity is induced as a response to oxidative stress, and AChE activity is specifically inhibited by organic pesticides such as organophosphates and carbamates. The concentrations of major contaminants (trace metals, polychlorinated biphenyls and polycyclic aromatic hydrocarbons) accumulated in the mussels tissues were also measured. Mussels from the most polluted sites consistently exhibited a significantly higher GST activity compared to the control site ($p<0.05$); whereas AChE activity was significantly inhibited. Both biomarkers showed the same pattern of GST induction and AChE inhibition during the two sampling years. The GPx activity was occasionally induced, but the pattern of response was less consistent that for the other two biomarkers. The most intense biological responses were recorded in the inner parts of the estuaries, where the most polluted areas were located. The above results suggest that GST and AChE activities can be used as potential biomarkers of toxicity for active monitoring in marine coastal ecosystems.

TU 381

Impact of suspended particles on bioavailability of petrogenic PAH in cod (Gadus morhua), mussels (Mytilus edulis) and passive samplers exposed to produced water

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Caged organisms and passive samplers are routinely used to monitor environmental impact of operational discharges from offshore oil and gas production. Comparison of exposure situations over time may be required. Sorption of Produced Water related hydrocarbons to particles in the sea water may influence the bioavailability of these compounds. Temporal variations of microalgae density may therefore affect exposure measurements in caged organisms and passive samplers. In order to investigate the consequence of differences in biological particle density on bioavailability of petrogenic polycyclic aromatic hydrocarbons (PAHs), a laboratory study was conducted in blue mussel (*Mytilus edulis*), Atlantic cod (*Gadus morhua*) and passive samplers (SPMDs) exposed to PW. Organisms and SPMDs were exposed for 17 days to diluted PW in a continuous flow-through system. The dilution was set at 0.1%, from day 6 until day 10 the flow was set to 0.2% to mimic real field conditions with varying PW concentration over time. The exposure set up included: a negative control with sea water only, a positive control with diluted PW only and three tanks containing diluted PW and algae mix at low, medium and high concentrations. The following parameters were analysed: PAHs in water, PAH concentrations in SPMD and mussel soft tissues, and PAH metabolites in fish. Due to the important role of gills for absorption of waterborne PAHs, a histophatological investigation of fish gills was conducted as a support parameter. The presence of realistic densities of organic particles had only minor impact on the bioavailability of low molecular weight PAHs in fish, mussels and passive samplers. Bioavailability of 3-ring PAHs was only significantly reduced in mussels when algae particle density was high (about 50000 cells/ml).

TU 382

Estimation of experimental conditions to maximize mussels shell capability in trace metals accumulation

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The evaluation of mussels shell capability of concentrate heavy metals was performed by means of a set of laboratory experiments conducted with mussels collected in unpolluted sites exposed to sub-lethal concentrations of a known toxic metal. Multiple cycles of accumulation and depuration were carried out all along the experimental period; the constant monitoring of experimental conditions and periodical metal determination in shell and tissue samples allowed the individuation of the accumulation and depuration patterns in both Bivalves materials in the selected species. Alternative exposure to contaminated and clean seawater respectively was performed in order to record the different metal concentration trends in shell and soft tissues. In fact it is demonstrated that once the metal is incorporate in the crystalline calcium carbonate lattice, his loss from the shell during mussels lifetime can be considered of no concern; in the other hand it is known that soft tissues are able to release contaminants in response to changed environmental conditions. The aim was to establish a set of experimental conditions able to optimize metal accumulation in mussel hard parts and to calculate the specific MATC (Maximum Acceptable Toxic Concentration) value. So it was possible to settle physical, chemical and physiological parameters in order to maximize the shell metal content and set the basis for implementation of bioinertization strategies exploiting mussels metabolic activity and detoxification mechanisms.

TU 383

The effect of sublethal nickel concentrations on Mytilus galloprovincialis: a multiple biomarker study

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Nickel is a known mammalian carcinogen and mutagen, typically found at low concentrations in the marine environment (<1 µg l-1), but enriched at coastal locations near mining operations. This study used a multiple biomarker approach to investigate the toxicity of sublethal concentrations of nickel to Mytilus galloprovincialis, a key bioindicator organism. Mussels were exposed to sublethal concentrations of nickel (0-3600 µg l-1) for 5 days. In addition to determining tissue-specific nickel accumulation, the biomarkers analysed were DNA strand breakage (comet assay; a biomarker of exposure), induction of micronuclei (a biomarker of effect), histopathology and expression of several key genes known to respond to oxidative or toxicological stress. Nickel accumulated in mussels at all concentrations, in the tissue-specific order byssus > digestive gland > gills > foot > adductor muscle > mantle. Significant genotoxic damage was observed by both the comet assay and micronucleus test, but only at 3600 µg l-1 nickel. Correspondingly, semi-quantitative analysis of histopathology in the gills indicated an increase in structural damage at concentrations ≥ 1800 µg l-1 Ni. Quantitative RT-PCR revealed overexpression of the glutathione-s-transferase (gst) and metallothionein (mt20) genes in gill tissue after exposure to 3600 µg l-1 nickel, compared to the controls. Expression of these genes is known to be induced following xenobiotic exposure, especially to metals. The p-glycoprotein (pgp) gene, which has been implicated in multi-xenobiotic resistance (MXR), was markedly overexpressed in all nickel exposed mussel gills, with the highest rate of expression at 18 µg l-1 Ni. These results suggest that nickel is genotoxic to mussels at 3600 µg l-1 and that this genotoxicity correlates with responses at the molecular (i.e. biomarker of exposure) and tissue (i.e. biomarker of effect) levels. As a result, we recommend that effort is made to accurately quantify nickel concentrations in highly polluted waters near nickel mines (e.g. the New Caledonian lagoon), and that remediative action may be needed if values exceed 3600 µg l-1.

TU 384

Biomarkers responses of the native clam Scrobicularia plana and changes of macrobenthic functional composition in a shallow tidal creek affected by fish aquaculture effluents

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The effects of solid organic wastes from a marine fish farm on sediments were tested using biomarkers in native clam (Scrobicularia plana) as biochemical indicators and macrobenthic community as ecological indicators. The clams and macrobenthic samples were collected in the intertidal sediment in October 2010 from five sites of the Rio San Pedro creek, following a gradient of contamination from the aquaculture effluent to the control site. Phase I and Phase II detoxification enzymatic activities (ethoxyresorufin O-deethylase (EROD), glutathione S-transferase (GST)), antioxidant enzymatic activities (glutathione peroxidase (GPX), glutathione reductase (GR)) and oxidative stress parameters (Lipid Peroxidation (LPO) and DNA strand breaks) were measured in clams' digestive gland tissues. Numbers of species, abundance, richness and Shannon diversity indexes were the biodiversity indicators measured in macrofauna. In parallel, redox potential, pH and organic matter in sediment, and dissolved oxygen in the interstitial water were measured in situ. Significant ($p < 0.05$) increases of GPX enzymatic activity, DNA damage and LPO, together with significant ($p < 0.05$) decrease of biodiversity indicators were observed in the areas close to the aquaculture effluent. Biomarkers (DNA, LPO and GPX) were significantly ($p < 0.01$) negative correlated with pH, redox potential and dissolved oxygen and positively correlated with organic matter. On the contrary, macrobenthic biodiversity were significantly ($p < 0.01$) positively correlated with pH, redox potential and dissolved oxygen and negatively correlated with organic matter. It has been demonstrated that effluents from fish aquaculture activities in Río San Pedro creek may induce oxidative stress in soft-sediment species which may lead to alteration of the biodiversity and health status of the exposed organisms.

TU 385

Mechanisms of acute toxicity of metals in estuarine and marine invertebrates

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In freshwater invertebrates, acute metal toxicity is generally associated with the disruption of ionic and osmotic regulations. In contrast, the mechanisms involved in acute metal toxicity in estuarine and marine invertebrates are not well characterized. Therefore, in the present study the most recent information generated by our research group on the possible mechanisms involved in the acute toxicity of metals in these animals was reviewed. Data on the acute effects of metals (copper, silver, zinc, and nickel) on biochemical and physiological biomarkes in estuarine and marine invertebrates (foraminifers, sea urchins, sea anemones, sea cucumbers, copepods, isopods, shrimps, crabs, and clams) were considered. Biomarkers evaluated included those associated with ionic and osmotic regulation of body fluids (ion and osmotic concentration and activity of associated enzymes), respiration (oxygen consumption), cellular oxidative status (enzymatic and non-enzymatic agents from antioxidant defense system), and energy metabolism (activity of enzymes from glycolysis and Kreb´s cycle). As observed in freshwater invertebrates, data available for the different groups of estuarine and marine invertebrates investigated indicate that ionic and osmotic imbalances can be observed when euryhaline animals are acutely exposed to metals in brackish waters, especially in very diluted salt water. However, no alterations have been observed when these animals are exposed to the metals in marine waters, suggesting that another mechanism might be involved in the acute toxicity of dissolved metals other than the imbalance of ionic and osmotic regulations, except for nickel. In this case, oxidative stress, as well as respiratory and energy metabolism impairments should be considered as potential mechanisms of acute metal toxicity in estuarine and marine invertebrates.

TU 386

Will biotic and abiotic factors increase the toxicity of environmental contaminants in exposed sea snails?

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Historically, mercury contamination has received an extensive attention due to its impact on human health and wildlife. Currently, concerns about climate changes and the way they might affect the environment are being more and more studied. Moreover predator-prey relations have been progressively more pointed as stress sources. Environmental contamination and biotic and abiotic stress co-occur, thus it is important to assess and understand the synergistic or antagonistic effects of these changes (e.g. salinity) and/or predation combined with common environmental contaminants.

Kairomones are signs produced by the predator and that can be detected by the prey, causing stress and consequent fitness costs. In order to measure those costs and evaluate the effects of simultaneous exposure to multiple stressors, the sea snail Gibulla umbilicalis was exposed to increasing concentrations of mercury and salinity and/or predatory risk, simulated by a concentration gradient of kairomones produced by a sea snail predator - sea star. Mortality, feeding rates (indirect measure by post-feeding exposure egestion) and avoidance behaviour were assessed. In the end of the experiments, animals were sacrificed and cholinesterases' activity and energy reserves were measured. Our results have shown that although responses could be triggered by single stressors, the combination of contaminants with abiotic or predator-specific compounds induces

stronger responses.
Studying both types of stressors and its interaction with contaminants can be crucial to provide improved interpretation of ecological effects of relevant scenarios for environmental risk assessment.

TU 387
Effects of mercury contamination on acute and chronic parameters of exposed sea snail Gibbula umbilicalis
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The Minamata (Japan) incident in the 1950's triggered the global concern regarding the mercury contamination hazard. Mercury is a very common contaminant in the environment, having natural sources, such as volcanism, and anthropogenic origins, such as mine tailings or industrial effluents.
Exposure to some environmental contaminants, even for short periods of time, may cause serious damage to exposed individuals. Thus, to evaluate the possible effects of mercury in the sea snail Gibbula umbilicalis, acute and chronic assays were performed by exposing the animals to increasing doses of the contaminant for 96 and 168 hours. Mortality, feeding rates (indirect measure by post-feeding exposure egestion) and avoidance behaviour were assessed. In the end of the experiments, animals were sacrificed and cholinesterases' activity and energy reserves were measured.
Although evaluated at individual level, the effects observed here might be translated to the population and community level, since feeding impairment and change in energy allocation may have effects on the organisms' fitness and reproductive performance. Moreover, modification in cholinesterases' activity and its common link to behaviour shifts might lead to severe implications in population dynamics.
Results have shown that most of the parameters evaluated tend to be impaired by this common contaminant, showing the deleterious effects that this compound may have at the population level and on marine life.

TU 388
Investigation on differential sensitivity of marine mollusk to various doses of detergent
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The problem of anthropogenic impact on marine aquatoria is one of the most actual nowadays. Synthetic detergents has been detected in municipal wastewater effluent and surface waters at different concentrations ranging from mkg/l to low mg/l. Detergents occur in the environment as complex compounds and could cause potential risk for humans and aquatic organisms. Few chronic ecotoxicological data are available with respect to detergents' effects in mussels. The critical issue is the choice of methods to determine concentration response threshold and the level at which detergents are essential for organism health status.
Objective of the present study was to develop improved method for assessing potential effect of detergent agent (e.g., SDS - dodecylsulfate of sodium) on marine mussels' cardiac activity and behavior. The study was conducted on adult 3-years mussels Mytilus galloprovincialis Lam. Continuous recordings of the heart rate and valve movements in the mussels were carried out in the laboratory under nearly natural conditions with running ambient seawater and addition of detergent. Previously, there had been reported that concentration of 1,7 mg/l detergent is the threshold for mussels to change their filtration activity. Our results have indicated that concentration of 0.5mg/l is the detection threshold limit of SDS in mussels, which could affect on heart rate and behavior (valve gape). In high concentrations detergent caused acute toxic action, which lead to rapid valve closure, isolation and subsequent bradycardia. Results have proved that implementation of behavioral and physiological biomarkers simultaneously could be effective tool to early detection stress exposure effects in marine organisms.
The study was partly supported by RFBR grant N 08-04-92424-BONUS_a.

TU 389
Biomonitoring trace metals in Mytilus galloprovincialis off the west coast of the Cape Peninsula, Cape Town
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The concentration of trace metals (Fe, Mn, Cu, Zn, Cd and Pb) in the mussel (*Mytilus galloprovincialis*) were measured along the west coast of the Cape Peninsula, Cape Town, from autumn 2010 to autumn 2011. Sampling took place at Scarborough (considered an unpolluted site), Hout Bay, Green Point, Milnerton and Bloubergstrand. The samples of whole animal soft tissue were analysed using an Atomic Absorption Spectrophotometer (AAS). Metals in *M. galloprovincialis* were significantly lowest at Scarborough and at the other sites differed significantly between seasons, with winter having the highest significant difference. The efficiency of metal accumulation was measured using the Biosediment Accumulation Factor (BSAF). The results showed that the BSAF was highest in Cd, Pb, Zn and Cu, with the lowest BSAF reported in Fe and Mn.

TU 390
PAM chlorophyll a fluorometry, field and laboratory research for corals and seagrass along the coast of Qatar
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A two year-long field program was conducted to evaluate the effectiveness of PAM (Pulse Amplitude Modulation) chlorophyll a fluorometry in monitoring the health of coral reefs and seagrass along the coast of Qatar. The technique was calibrated and validated as a means of assessing sub-lethal stress for coral in the region. The reduced photosynthetic efficiency indicated by the PAM, without clear visual signs of impairment in coral structure, was generally followed by necrosis of parts of the colonies; this was visually verified in subsequent campaigns. Two PAM derived measurements; photosynthetic efficiency and Rapid Light Curves (RLC) were well correlated with visual observations on the health conditions of the organisms. Furthermore, this work has suggested a critical threshold value applicable to photosynthetic efficiency for corals in this environment. For seagrass, there was correlation between RLC data and visual observations, such as high epiphytic coverage; however, PAM measurements did not appear to provide as strong an indication of sub-lethal stress before deterioration as obviously as it does for corals. This research has now been integrated with laboratory studies utilizing both the diving PAM used in field research and the imaging PAM, which can measure photosynthetic processes and obtain detailed visual images of photosynthesis. Gulf coral collection and cultivation and its detailed monitoring were the first steps of corals (and seagrass) laboratory experimentation. PAM measurements were performed on the Acropora sp. colonies prior to and after collection. "Mother"colonies were tagged and photographed to document their recovery. PAM measurements were continued in the laboratory in order to monitor the recovery of the nubbins after collection and transfer. Protocols for future coral experiments are being tested and adapted. Laboratory experimentation is also underway on seagrasses to better understand the driving factors that influence sustained photosynthetic performance for this important ecological resource.

TU 391
Toxicity tests using bioluminescent Phytoplankton
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In the field of risk assessment of chemicals entering the marine environment, tools are needed as the pressure of several pollutants impacts marine and estuarine ecosystems. Relevant sources may be the chemicals from gas/oil production platforms and sea-side refineries. The discharge of compounds to the marine environment will be receiving additional attention in the EU Marine Strategy and rapid assessment tools are needed to identify and prioritize sources. Most recently, the disinfection of ballast water will be significant interest as the International Convention for the Control and Management of Ships' Ballast Water and Sediments will introduce worldwide requirements for treatment standards. Most of these treatment systems use chemicals; and therefore risks need to be identified before market (certification) and after (monitoring and control). Therefore, a rapid toxicity method to determine the effects of contaminants in marine waters has been developed and used in such tests. This innovative test method uses bioluminescence from marine dinoflagellates, and can serve as a quick and easy to use test to screen for effects in marine waters. The test is ASTM Standard E1924 "Standard Guide for Conducting Toxicity Tests with Bioluminescent Dinoflagellates". The QwikLite(R) toxicity system is a rapid, cost effective, portable bioassay based on inhibition of light produced by marine bioluminescent dinoflagellates. The bioluminescent species *Pyrocystis lunula* are used according to their robustness and sensitivity for various toxic substances in the environment. Currently, the method using the dinoflagellates is pilot tested in the marine laboratory. The first results on reference compounds and environmental samples will be presented.

TU 392
Development and validation of a colorimetric methodology to assess macroalgae chronic toxicity - a case study with Ulva lactuca
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Xenobiotics are increasingly present in everyday life and are easily integrated into marine ecosystems. In aquatic systems macroalgae are continuously exposed to

contaminants. Since they represent a very large biomass, they can act as an important sink for contaminants becoming a gateway for higher trophic levels.
Moreover, it is known that macroalgae, especially estuarine, are very important in the aquatic system due to several aspects: easy to find and to identify, vast biomass percentage and great bioindicators, making them potential sentinels of to monitor xenobiotics in aquatic environments.
Macroalgae presence or absence, as well as their look-like may reflect the current state of the marine ecosystem, thus it is mandatory to develop new tools and methodologies to quickly and cost-effectively assess their status.
The green macroalgae Ulva lactuca was used as a model species. The macroalgae, when exposed to xenobiotics, can show signs of decay by losing their colour and their texture. But these signs only are assessed by visual inspection. To be sure the algae are in decay and correctly quantify it, a new method was developed by measuring the colour across a colour scale. For this a colorimeter (Chroma Meter CR 400/410- Konica Minolta) was used and color variations calculated when exposed to contaminants. Also its validation by measuring photosystem electron transfer in exposed macroalgae was performed.
The methodology revealed to be a sensitive and effective measure of xenobiotic toxicity and a prospective tool for environmental risk assessment.

TU 393
Analysis and environmental distribution of organic micropollutants in urban protected salt marsh areas
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Coastal ecosystems concentrate most of the marine organisms and species compared to open ocean areas. However, they are often heavily affected by human activities that can jeopardize their populations. Therefore, contamination by organic compounds discharged by nearby industries and cities has attracted considerable attention from multiple perspectives. Our first objective in this work was to develop a fast, easy and low-solvent consumption multiresidue method for the simultaneous identification and quantification of a wide variety of classic and emerging pollutants that often impact coastal marine systems. Stir bar sorptive extraction (SBSE) and liquid desorption (LD) coupled to gas chromatography tandem mass spectrometry (GC-MS/MS) were used for determining polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), organochlorine and organophosphorus pesticides, triazines, plasticizers and some personal care products (triclosan, UV filters and fragrances). Briefly, 10 mm stir bars coated with polydimethylsiloxane (PDMS) were placed in a flask containing water samples (100 mL) and stirred for 24 hours at room temperature. Then, bars were sonicated during 30 min using a reduced amount of ethyl acetate (0.2 mL) to release the analytes. This extract was analyzed by GC-MS/MS, so sub-ppb levels could be detected. Several parameters were optimized: extraction and desorption time, ionic strength, presence of organic modifiers, pH and MS/MS transitions. The second objective was applying this method to characterize the presence and distribution of micropollutants in two protected salt marsh areas from SW Spain that are adjacent to cities (Cadiz and Huelva, respectively). 20 water samples were collected at each area during summer 2011. Concentrations were often below 1 µg/L for most analytes; nevertheless some of them such as triclosan (up to 0.3 µg/L) can act as endocrine disruptors and affect marine organism populations after chronic exposure. Overall, results from this study provide an overview on the state of the two sampled marine environments with respect to chemical pollution, being useful for carrying out future toxicological assays with native species.

TU 394
Mixture toxicity of anthropogenic and natural compounds to marine micro-algae
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Primary production by micro-algae embodies the carrying capacity of marine ecosystems and is primarily linked to nutrient availability and light. However, recent studies indicate that certain industrial chemicals may have a direct impact on coastal plankton communities and hence on the carrying capacity of estuarine and marine ecosystems. At the same time the frequency and intensity of toxic algal blooms in the coastal zone are increasing globally, resulting in increased levels of natural toxins expecting to affect coastal ecosystems. These different chemical stressors are hypothesized to disturb regulatory mechanisms within algal communities, modifying the competitive abilities of individual species and resulting in shifts from highly nutritious to unfavourable algal species that destabilize the food chain. However, insight in the potential antagonistic or synergistic action of toxicants and toxins is lacking, which may mask or enhance the toxic effects. Therefore this study aims to provide information on the combined toxicity of both type of compounds to a range of marine micro-algae. Three algal species (*Dunaliella tertiolecta*, *Phaeodactylum tricornutum* and *Thalassiosira pseusodonana*) were exposed to two anthropogenic compounds (Irgarol and Tributyltin) and two natural toxins (Decadienal and microcystin). For *D. tertiolecta* binary equitoxic mixtures of the anthropogenic compounds and the natural toxin Decadienal were tested according to the Toxic Unit (TU) concept. Reduction in effective photosystem II (PSII) efficiency was determined by Pulse Amplitude Modulation (PAM) fluorometry after 4.5 hours as a measure for toxicity. A difference in species sensitivity was observed with *T. pseudonana* being the most sensitive species. Irgarol is the most toxic compound for all tested species and the natural toxin Microcystin is the least toxic. Both natural toxins are clearly less toxic to the tested algae than the two anthropogenic compounds. A small, less than additive mixture effect was observed for Decadienal and TBT, while a large more than additive mixture effect was found for Decadienal and Irgarol. These results suggest that single compound toxicity data can not be used to predict the mixture toxicity of these compounds. A species, compound and mixture specific response was observed which underlines the complexity of determining the toxic pressure of coastal waters on the primary producers.

TU 396
The use of protozoa in ecotoxicology: from biological model to environmental biomonitoring application
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Nowadays, anthropogenic activities have resulted in accumulations of inorganic and organic pollutants in the environment as well as in the food chain, hence leading to serious ecological and human health problems. This may pose a risk to benthic and epibenthic organisms and it is crucial to discover tools that will identify adverse effects of sediment-associated chemicals on benthic organisms as well as link toxicity mechanisms to biological effects. Due to their nature as a eukaryotic cell/organism and their position in the food web, ciliated protozoa are suitable models for evaluating the biological effects of chemicals in living organisms as well as in estimating pollution levels in aquatic environments. Lethal and sub-lethal effects of exposure to inorganic and organic pollutants were tested on the cell mortality, replication rate, lysosomal membrane stability and endocytosis rate of the marine ciliate E. crassus. Increasing concentrations of mercury, copper, and benzo(a)pyrene , individually and as mixtures, were investigated in our study as they might be bioavailable in naturally occurring polluted sites. Exposures to binary mixtures of all studied pollutants were performed showing both inorganic-organic and inorganic-inorganic additive and/or antagonist effects. Moreover, medium salinity was also varied to mimic estuarine-like environmental conditions linking biological response to ionic strengths. Results of laboratory experiments underlined that, due to the short time and simplicity of the test procedures, the use of protozoa such as E. crassus is a promising and convenient bioindicator for evaluating the toxicity of different environmental matrixes like pore water, sediments and wastewaters - polluted by heavy metals and organic pollutants. To validate the laboratory experiments a pilot biomonitoring program was performed in the Venice Lagoon, a peculiar, fragile ecosystem characterized by high economic and ecologic values. The responses of the battery of biological indexes in E. crassus were combined with sediment and elutriate chemical analyses. Overall outcomes were able to discriminate a gradient of stress in the four investigated sites. Furthermore, biological responses were linked to the specific occurrence of a certain class of pollutants in both sediments and elutriates.

TU 397
Can foraminifera be reliably used as environmental indicators?
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Sediment samples from around the Robben Island sewage pipeline and a fish factory pipeline in St Helena Bay were examined for foraminifera as well as for a suite of environmental factors. X-ray analysis was conducted on "live"foraminiferal tests to determine correlations with trace metals within the sediments. The species diversity, richness and abundance were negatively correlated with trace metals. The percentage nitrogen was negatively correlated with diversity while the percentage carbon was positively correlated with abundance and richness. The mean grain size was negatively correlated with richness, diversity and abundance, although these correlations were low. The percentage carbon, percentage nitrogen, and the mean grain size most influenced community structure. The dominant genera in St Helena Bay were negatively correlated with trace metals and percentage nitrogen. The dominant genera from around Robben Island were most strongly correlated with the mean grain size. Bolivina, Elphidium and Quinqueloculina appeared to be most strongly affected by environmental factors than the other genera and may be regarded as good bio-indicators. Ammonia, although dominant was less correlated with environmental factors and because it is considered an opportunist.
No significant correlations could be found between the trace metal concentrations of the tests and the sediments. It appears that foraminifera can regulate the concentration of trace metals within their tests, evident in the low concentrations of trace metals in tests despite high concentrations in the sediments. St Helena Bay samples also displayed a higher concentration of trace metals but a lower Ca:Mg ratio than those of Robben Island. Trace metal concentrations appear to interfere with the laying down of calcium carbonate within foraminiferal tests.
Despite variability and patchiness normally found in benthic meiofauna, trends found in foraminiferal assemblages could be related to environmental conditions, particularly the trace metal concentrations within the sediments. Despite the trace metal tests not being significantly correlated with the trace metals in the sediments, trace metals do appear to have an effect on Ca:Mg ratios within the tests.

TU 398
The effect of a Copper and Zinc mixture on Dunaliella tertiolecta
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Toxicity experiments are usually performed with one compound, but contamination in the field generally consists of mixtures of toxicants. The aim of this study was therefore to determine the effect of a Cu+Zn mixture on the marine alga *Dunaliella tertiolecta*. Growth rate (cell count) and photosystem II efficiency (Pulse Amplitude Modulation fluorometry) of *D.tertiolecta* were determined after three days of exposure to Cu, Zn and equitoxic mixtures of Cu+Zn, composed according to the Toxic Unit concept. Our results showed that growth of *D. tertiolecta* was more sensitive to Zn than to Cu. Photosystem II efficiency was less sensitive to the metal(mixtures) than growth. The relative

insensitivity of photosystem II efficiency to these two metals requires determination of growth inhibition in order to prevent underestimation of metal(mixture) toxicity. The toxicity of the mixture of Cu+Zn was more than concentration additive and could thus not be predicted based on single metal toxicity. Hence, a reliable estimation of metal toxicity requires experimental verification of predicted effects.

TU 398

Toxicological effects of three polybromodiphenyl Ethers (BDE-47, 99 and 154) on growth of marine algae Isochrysis galbana

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Polybrominated diphenyl ethers (PBDEs) are highly persistent anthropogenic contaminants found in trace amounts in many environmental compartments far from their source areas, posing a risk to aquatic ecosystems. Our objective was to determine the relative toxicities of three BDE congeners such as BDE-47, 99 and 154 on marine phytoplankton algae Isochrysis galbana. For a highly sensitive endpoint: a 72h-inhibition of autotrophic growth rate was calculated according to standards methods. No observable effect concentration (NOEC) values were 2.53, 3.48 and 12.3 µg L-1 for BDE-47, 99 and 154, respectively and LOEC values were 5.06, 6.96 and 24.60 µg L-1 for BDE-47, 99 and 154, respectively. The calculated IC10 (the concentration inhibiting growth rate by 10%) corresponded to 9.3, 12.78 and 54.6 µg L-1 for BDE-47, 99 and 154, respectively. The 50% inhibitions of growth rate (IC50) values were: 25.7 µg L-1 BDE-47, 30.0 µg L-1 BDE-99 and 243.7 µg L-1 BDE-154. Therefore, the acute toxicity of PBDEs decreases as the degree of bromination increases, the order of toxicity being BDE-47> BDE-99> BDE-154. Significant ($p < 0.05$) adverse effects were observed for all compounds at concentrations >15 µg L-1. Our results indicated that under laboratory conditions PBDEs were acutely toxic to seawater algae at concentrations near 10 µg L-1. However, further work is required to investigate long-term effects in these and other aquatic organisms.

TU 400

Tissue accumulation of fluoranthene and its metabolites in the shore crab Carcinus maenas in relation to oxidative stress

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Concern around contamination of the aquatic environment by polycyclic aromatic hydrocarbons (PAHs) is high due to their toxic, carcinogenic, mutagenic and/or teratogenic effects. Fluoranthene (FLU) is a priority PAH that is commonly detected in sediments, water and biota of European estuaries. It is persistent and an important lipophilic constituent of crude oil. Early detection of environmental exposure and effects of FLU on marine organisms is thus an issue of utmost relevance. *Carcinus maenas* is a natural inhabitant of European coasts and estuaries. It is a suitable sentinel species widely used to assess exposure to contaminants, including PAHs. This work investigated the accumulation of FLU and its metabolites in the tissues of *C. maenas* following a 7-day laboratory exposure. Effects of FLU on oxidative stress biomarkers were also investigated. Crabs were collected from a low impacted estuary. In the lab they were acclimated (7d) in filtered seawater (15 psu; 16ºC) and exposed individually (9 per treatment) to 5 FLU concentrations between 1.90-75 µg/l. At the end of the bioassay, crabs were ice-anaesthetized and samples of digestive gland were collected for biomarker determinations: glutathione-S-transferases (GST) and glutathione reductase (GR) activities, levels of total glutathione (GT) and lipid peroxidation (LPO), and FLU-type metabolites as measured by fixed-wavelength fluorescence (FF). The remaining whole soft tissues were used for chemical analysis of FLU by GC-MS. The results indicate a strong concentration-dependent accumulation of FLU (measured by GC-MS, ß = 5.21, $P < 0.001$, R2 = 0.993) in the soft tissues and FLU-type metabolites (measured by FF, ß = 6.09, $P = 0.002$, R2 = 0.931) in the digestive gland. A strong positive linear relationship (ß = 1.15, $P = 0.003$, R2 = 0.911) between accumulation of FLU (GC-MS) and FLU-type metabolites (FF) was also found. GST activity and TG levels were significantly increased in crabs exposed to concentrations ≥12 µg/l, compared to controls. GR activity was significantly increased, and LPO was decreased, in crabs exposed to concentrations ≥30 µg/l, relatively to controls. In conclusion, accumulation of FLU and its metabolites appears to be a useful biomarker of exposure and bioavailability of this PAH to C. maenas.

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TU 400

Comparison of proteomic profiles in gills of shore crabs (Carcinus maenas, L.) from urban and reference sites and variation of polyubiquitinated proteins

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Coastal waters adjacent to urban and industrial zones can be deeply impacted by chemical pollution that represents a threat for the health of resident organisms. Pollution-induced alteration of cellular processes notably includes the modulation of protein metabolism and homeostasis, so that proteomics can help to understand the sub-lethal effects of chemical stress. Especially, the study of protein ubiquitination provides a particular insight into the effects of environmental stress in terms of damages to biomolecules and regulation of degradation systems as well.

We investigated the response to environmental stress in *Carcinus maenas* (L.) by comparing 2-DE proteomic profiles and protein ubiquitination in gills of crabs sampled in a polluted site and a reference site at low tide. Proteomic profiles showed significant differences according to animal origin despite high individual variability. A small set of regulated spots (Student's t-test, n=7, $p<0.05$, 20% FDR) successfully classified the two sites by hierarchical clustering, confirming the discriminatory power of gill proteomic pattern. Immunoblotting of polyubiquitinated proteins with polyclonal antibody and ECL detection enabled to visualize 20 spots in average. In spite of the high individual variability of the pattern, two spots exhibited significantly different ubiquitination levels between conditions (Mann-Whitney U test, n=3, $p<0.05$).

These results highlight that protein ubiquitination assessment represents a promising approach to evaluate and gain insight into stress response in marine ecotoxicology. Proteins whose expression and/or ubiquitination level are affected by crab location will be subjected to identification by mass spectrometry, in order to tentatively elucidate the mechanisms involved in pollutant response.

TU 401

Does contamination impact the shore crab (Carcinus maenas L.) resistance to an additional stress? An insight through a multiparametric approach

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The shore crab *Carcinus maenas* (L.) represents the most common crab species and a key component of littoral ecosystem in european shores. The wide geographical distribution of populations, their presence in pristine as much as heavily polluted areas and their intertidal location reflects a remarkable ability to cope with different stresses, due to a great physiological plasticity notably. However, the compensation capacities that underlie tolerance to stress may be impacted by the life history of crabs in terms of exposure to pollutants notably. In addition to the chronic exposure to contaminants, a novel insult is indeed likely to generate a too high degree of stress that would overwhelm resistance capacities of organisms.

To test wether and understand how chronic exposure to pollutants affects the resistance capacities of *Carcinus maenas* to stress, we imposed a 4-days air exposure to crabs sampled in a polluted area, Le Havre harbour, and in a clean area, Yport rocky shore (France) and analysed stress markers as well as proteome variations in gills. For each site, antioxidant activities (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione S-transferase), protein expression (2D-electrophoresis, SDS 12% polyacrylamide gels, pH 3-10) and polyubquitination (immunoblotting of 2D-E gels, ECL detection) profiles of air-exposed crabs were compared to those of control individuals that were continuously kept in water. Crabs from Le Havre showed a higher mortality rate (5%) than crabs from Yport (0%) in the course of the experiment, but no difference was found between air-exposed and control groups whatever the sampling site. Results indicate an altered resistance capacity in crabs living in Le Havre harbour in comparison with those from the clean site Yport.

Proteins showing different expression or polyubiquitination levels will be identified by mass spectrometry in order to elucidate the mechanisms underlying stress response. We intend to further investigate the response to prolonged air exposure in a less resistant crab species, the flying velvet crab *Necora puber* (L.), sampled in the same sites as *C. maenas* in order to compare the physiological and cellular responses to stress between the two models.

TU 402

Effects of mercury in natural populations of the sea anemone Actinia equina

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Mercury (Hg) is considered to be one of the most toxic metals to biota, being capable of biomagnification and bioaccumulation in food chains. High concentrations of Hg were associated with developmental and behavioral abnormalities, impairment in growth and reproduction, and reduced survival. Though this metal has been extensively studied, several knowledge gaps still exist, mainly regarding its effects in different types of organisms. Accordingly, this study aimed at assessing the effects of a short-term exposure to Hg in natural populations of the sea anemone Actinia equina. To attain this objective, four natural population of A. equina were sampled in the NW Atlantic coast. From each population, forty individuals were collected and acclimated in laboratory, under optimal conditions, for one month. Afterwards, organisms were weighed (7.09 ± 0.18 g mean ± SE), and using a flow-through system were exposed to 100 µg L-1 of Hg and to artificial sea water solely, during 96h. At the end of exposure, organisms were immediately deep-frozen (-80°C) until further analysis. The tissue of pedal disc was analyzed for bioaccumulation and biomarkers (biochemical: glutathione S-transferase-GST, catalase-CAT and lipid peroxidation-LPO and metabolic: lactate and alanine) to compare the uptake, oxidative damage and metabolic patterns among populations after being exposed to Hg. All population exhibited significant bioaccumulation of Hg and a significant change in the activity of LPO when exposed to Hg. On the contrary, exposure to Hg caused no significant changes in the activities of GST, CAT and in the levels of alanine and lactate. Furthermore, significant differences in the response patterns to Hg among the four populations were observed. With the two populations originated from sites with a higher Hg contamination showing a lower bioaccumulation of Hg and a higher oxidative stress. Also, the ratio of lactate/alanine (an index of redox state of the cell) accomplished with the biochemical biomarkers responses revealed that those two populations exhibited a lower stress redox. This findings suggest that the use of several endpoints at different levels of biological organization is the important for the evaluation of Hg effects in the biota.

TU 403

A short-term in situ sediment assay based on the postexposure feeding of the estuarine isopod Cyathura carinata

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In situ assays based on feeding depression have been proposed as sublethal assays which allow to assess direct and immediate contaminant effects on key ecosystem functions (e.g. organic matter decomposition, grazing), long before direct effects on individuals (e.g. growth) are extrapolated to effects at higher levels of biological organization - the traditional approach when selecting sublethal test endpoints. In situ assays provide a more realistic exposure scenario than assays conducted under laboratory controlled conditions, which is particularly relevant for estuarine ecosystems where environmental conditions are highly variable. In this context, the objective of the present study was to develop and evaluate a short-term cost-effective in situ sediment assay based on the postexposure feeding of an estuarine species widely distributed and with an important ecological role in estuarine foodwebs. The selected species was the benthic isopod *Cyathura carinata*, a secondary producer, prey to fish and birds, occurring along the North Atlantic coast and in the Baltic and Mediterranean seas. The methodology for feeding quantification was first developed and optimized under laboratory conditions with *Artemia franciscana* (Crustacea) nauplii. Then, the sensitivity of the postexposure feeding response to a reference chemical - cadmium - was compared to that of lethality, by conducting tests during a 48-h exposure period. Finally the in situ postexposure feeding assay was evaluated by deploying organisms in chambers at reference and contaminated Portuguese estuaries (Mira and Sado Rivers, respectively). The influence of different exposure conditions (sediment particle grain size, temperature, salinity, and food availability) was also taken into account through a multiple regression model, with the ultimate goal of discriminating contaminant effects from those due to environmental factors. The in situ postexposure feeding assay with *C. carinata* was found to be a potential useful cost-effective tool for estuarine sediment toxicity assessments.

TU 404

Toxicity of the biotransformation products of four methylated PAHs and one PAH produced by Nereis diversicolor and Nereis virens

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Risk assessments of spilled oil generally focus on the *content* of specific PAHs in a contaminated site. When the content of these compounds are below the set limit, elimination of the contamination has succeeded. However, when spilled oil is biotransformed, a wide range of water soluble products are created. These compounds can reenter the sediment by deconjugation reactions, but can also act as toxicants in the water column. If these transformation products are toxic, they do not only expose organisms in the water, they are also subjected to spreading in the environment in a much larger scale.
The objective of this study is therefore to: 1) assess of the toxicity of the biotransformation products of 1-methylphenanthrene, 3,6-dimethylphenanthrene, 1-methylpyrene, 5-methylchrysene and phenanthrene produced by the two benthic invertebrates *Nereis diversicolor* and *Nereis Virens*, and 2) identify the most important water soluble transformation products.
The biotransformation products from PAH exposed *N. diversicolor* and *N. Virens* were collected by extracting the test water of laboratory mesocosms exposures at different times. The water soluble biotransformation products were extracted and concentrated by SPE, and their toxicity (EC50) were determined by exposing *Daphnia magna* to dilutions of these extracts. The water soluble biotransformation products were identified and quantified by UHPLC connected in series to a fluorescence detector and a Quadropole Time of Flight Mass Spectrometer. To aid the identification enzymatic deconjugation of the phase II products was performed, and identified by use of Gas Chromatography Mass Spectrometry (GC-MS).
This work presents a novel approach to test the toxicity of transformation products of a contaminant mixture, where organisms are exposed to the entire range of biotransformation products. Also, this work shows the changes in toxicities of the transformation products as transformation of the PAHs progresses, and gives insight to the mechanisms of biotransformation of alkylated PAHs.

TU 405

Vitellogenin and Zona Radiata gene expressions in three different species of catfish from Terminos Lagoon

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In an effort to assess the differences in the Vitellogenin and Zona Radiata gene expressions of three different species of catfish (Ariopsis felis, Bagre marinus and Cathorops melanopus) collected in the Laguna de Terminos, 70 fish were collected at 5 different sites of the lagoon and were dissected to obtain liver. Total RNA was extracted using the Trizol method along with purification with the DNase enzyme. Specific primers of Vitellogenin and Zona Radiata genes were custom designed to amplify and isolate two partial fragments from Ariopsis felis, Bagre marinus y Cathorops melanopus liver tissues. Gene expressions were analyzed using amplification with Reverse Transcripatase-Polymerase Chain Reaction (RT-PCR) and results showed statistical differences between Vitellogenin and Zona Radiata values for fish collected at Laguna de Terminos. Data from this experiment show that Vitellogenin gene expressions were more sensitive than the ZR, under normal conditions in the environment. Future studies will be focused on understanding the reproductive cycle and the genetic variability of these fish.

TU 406

Polybrominated diphenyl ethers and their methoxylated analogs in sea bass (Dicentrarchus labrax) from Bizerte Lagoon, Tunisia

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Concentrations of polybrominated diphenyl ethers (PBDEs) and methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in sea bass (Dicentrarchus labrax) collected from the Bizerte Lagoon and the Mediterranean Sea (reference site) were investigated. To the best of our knowledge, this is the first report of these compounds in marine fish from Tunisia. Concentrations of ∑PBDEs ranged from 37.3 to 218 ng g-1 lipid weight (lw) for sea bass in Bizerte Lagoon, whereas in the Mediterranean Sea, concentrations ranged from 20.8 to 36.6 ng g-1 lw. BDE-47 was the major congener in samples from the two areas. Mean levels of MeO-PBDEs ranged from 49.4 to 798 ng g-1 lw in samples from Bizerte Lagoon. In the Mediterranean Sea, the mean concentration of these naturally produced organobromines was 482 ng g-1 lw. The total PBDEs and total MeO-PBDEs concentration in sea bass from Bizerte Lagoon was similar or slightly lower than those reported for other species from other locations around the world. No significant correlations were found between the organohalogen compounds levels and the fish characteristics.

TU 408

Seasonal pollutant monitoring in discarded fish species: is valorisation appropriate?

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Increased by-catch utilisation interest may come from a greater demand for fish products: the development of new markets for previously discarded species, use of low-value by-catch specimens for aquaculture and animal feed or the creation of value-added products from by-catch or discarded fish for food, pharmaceutical or cosmetic industries. However, the contribution to a sustainable management of this biomass through their optimal valorisation highly depends on the quality of the products to be obtained from them.
Many studies in the scientific literature, like surveys of fish and fish products in markets of different countries [1] or monitoring reports of Public Administrations and the EU [2] reported significant levels of this kind of pollutants (especially of dioxins, polychlorinated byphenils-PCBs, organochlorinated pesticides-OCPs and heavy metals) in several cases for commercial species of different fisheries. Hence, it is logical to assume the presence of contaminants in other non-commercial species, although contamination levels in these non-targeted and/or discarded species are not usually assessed. However, a sustainable management of discards passes through the evaluation of their pollutant content, especially if it is considered that the products of discard valorisation are mainly concentrates (as fish oil and meal). Pollutants contained in the raw material are usually present at higher concentrations in the valorised product, especially if the product has a high fat content. In fact, some marine valorised by-products present pollutant levels of concern [3].
In this sustainability framework, FAROS project, co-funded under the LIFE+ Environmental Program of EU (LIFE08 ENV/E/000119), aims as one of its main objectives to analyze the valorisation potential of fish discards in order to contribute to their sustainable management by minimizing discards/by-catch through their optimal valorisation to recover and to produce valuable chemicals of interest in the food and pharmaceutical industry [4]. To properly define these adding-value processes, monitoring of metals, PCBs and dioxins are being performed on the most discarded species identified in FAROS métiers.

TU 409

First evidences of suboptimal health status of red mullets from the priority polluted Mediterranean area of Portman (SE Spain)

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The Portman area (Cartagena, SE Spain) is one of the areas most heavily polluted by metals in the western Mediterranean Sea and highly impacted by emissions from oil-related and chemical industrial activities in the adjacent Escombreras Valley. An integrated assessment was conducted of the health status of red mullet (Mullus barbatus) from three priority pollution areas of the Spanish Mediterranean coast, including Portman. A suite of general physiological indicators (lipid content in muscle tissue, condition factor, hepatosomatic and gonadosomatic indexes) and hepatic biomarker responses (ethoxyresorufin-O-deethylase (EROD) activity, metallothionein concentration and DNA damage) were determined. In addition, fish muscle tissue and sediment samples were taken and analysed for concentrations of arsenic, trace metals, Polychlorinated Biphenyls (PCBs), chlorinated pesticides (dichloro-diphenyl-trichloroethane (DDTs), α-hexachlorocyclohexane, lindane, hexachlorobenzene, cyclodiene insecticides and trans-nonaclor) and polycyclic aromatic hydrocarbons (PAHs). Our results showed that fish from Portman accumulated the highest concentrations of mercury, lead and arsenic and also of some PAHs and PCBs congeners. In addition, they had significantly lower lipid content, condition factor and gonadosomatic index, as well as the highest the highest levels of EROD activity and DNA damage of the areas investigated. Contaminant body burden in red mullet only partly corresponded to chemical characteristics of the sediments in the areas in which they were collected. Despite current concentrations of contaminants in sediment and fish muscle from the three priority pollution areas of the Spanish Mediterranean shelf investigated herein are at levels that do not give rise to severe health effects in red mullet, our findings indicate that fish from Portman exhibited a suboptimal health status that warrants further study. The integrative assessment strategies, including chemical, (eco)toxicological parameters used in this study can contribute

to the assessment of Good Environmental Status under the Marine Strategy Framework Directive.

TU 410
Characterization of selected Metals in United Arab Emirates coastal fish and locally produced vegetables
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Heavy metal pollution of the marine environment has long been recognized as a serious environmental concern. A lack of published literature on heavy metals contamination of local fish and a proper fish advisory in the United Arab Emirates (UAE) is in current need. Moreover, seafood and locally produced vegetables comprises a major portion of local daily consumption. An assessment of the contamination of selected metals including arsenic, cadmium, lead and mercury was made in several UAE food products such as fish, seafood and vegetables (sardine, shakelie, trevaly, mushrooms, cucumbers, peppers, among others) using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The concentration levels were dependent on the food type and metal tested. For example, arsenic was found in sardine and shakelie at concentrations of 0.0241 mg/kg and 0.0298 mg/kg, respectively. The lowest arsenic concentration was obtained in peppers at 0.089 mg/kg. This data provides preliminary information for the further formulation of health risk assessments for the community of UAE.

TU 411
Sediment-contact exposure of medaka eggs to a PAH model: combined chemical, toxicological and proteomic approaches
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Estuarine ecosystems represent nursery areas for many fish species. They are nevertheless submitted to a high anthropic pressure, which is manifested by pollution. Among the most important pollutants are polycyclic aromatic hydrocarbons. The main objectives of this study were to understand transfers between the different compartments during exposure and to see if the macroscopic effects observed were related to proteomic modulations. We spiked reference sediment with 3 concentrations of fluoranthene, then exposed Japanese medaka via direct contact with sediment during their entire embryonic development. Water, sediment and embryos were collected during the exposure and parent fluoranthene was analyzed by GC-MS. Time to hatch was also determined and photography was performed to analyze morphologic abnormalities and total body length. They were then sacrificed in liquid nitrogen to perform proteomic analysis by 2-dimensional electrophoresis. In sediment, the concentration of fluoranthene decreased linearly with time. In water, concentrations were globally constant, with the sediments acting as a reservoir of pollutant. In embryos, the concentrations were more erratic, and were dependent on stage of development more than concentrations in sediment or water with a fast clearance after hatching. At the same time, the highest tested concentration led to hatching failure associated with one day delayed hatching. The rate of several abnormalities increased such as heart edema, skeletal deformities, heart defects and abnormally reducing of yolk sac. These pathologies, related to blue sac disease syndrome, explained the dose-dependent increase of teratogenicity. The proteomic map exhibited 600 proteins. Among these, the expression level was modulated for 77 proteins. Cytoskeleton appeared to be regulated during exposure, as well as proteins related to cell physiology, fatty acid or protein metabolism and transport. Macroscopic and microscopic effects and/or regulations were dose-dependent, which suggest that fluoranthene is involved in a metabolic pathway that we could not determine with our proteomic study. Exposure to environmental concentrations of a single PAH in a realistic way of exposure could lead to proteomic modulations, with macroscopic teratogenic effects. This study was supported by region Haute Normandie and Aquitaine, GIP Seine Aval and University of le Havre.

TU 412
Long-term incubation of adult Nereis virens (Annelida: Polychaeta) in copper-spiked sediment: the effects on adult mortality, gametogenesis, spawning and embryo development
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The impacts of copper on polychaetes have received considerable attention with many sub-lethal endpoints developed including effects on reproduction. However, no reproductive experiments with large polychaetes lasting longer than a few days have been attempted. N. virens is an ideal candidate for long-term parental exposure investigations as gametogenesis and spawning of of a population are highly synchronized. Heavy metals interact with sediment in many ways and spiked sediment has been used frequently to aid in the development of concentration-response relationships. In this study we investigated the effects of copper on the later stages of the gametogenic cycle of N. virens through spiking sediment to a range of nominal concentrations (50, 500 and 1000 mg kg-1 dry weight of sediment) chosen to reflect those present in sediments experienced by macrofaunal polychaetes. Subsequent adult mortality and the numbers and timing of spawning events were quantified as well as the effects on oocyte development. Finally, sperm and oocytes were extracted from the adults and using in vitro fertilizations we assessed the developmental success. BCR 3-step standard sequential extraction confirmed that much more labile copper (in actual and percentage terms) was present as spiked concentrations increased, although the residual fractions had similar amounts and this is comparable to those levels found in the tissues of the exposed worms. Adult mortality was not dependent on the exposure time, but higher concentrations induced greater mortality for both sexes. Oocytes were significantly smaller at higher concentrations although pairwise comparisons did not show specific differences. Spawning of males was earlier and more frequent in the higher concentrations. Differences in the number of embryos developing normally after in vitro fertilizations of oocytes fertilized with sperm from exposed males and not exposed males showed that sperm were more susceptible, but oocytes were also affected at the highest concentration. Exposure of adults to copper may have significant implications for adult survival, embryo development and larval recruitment and subsequent colonization of polluted sediments.

TU 413
Oxidative stress, genotoxicity and histopathology biomarker responses in sea bass (Dicentrarchus labrax) liver from Bizerte Lagoon (Tunisia)
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The aim of the study was to evaluate the impact of environmental contaminants on oxidative stress, genotoxic and histopathologic biomarkers in liver of sea bass (Dicentrarchus labrax) collected from a polluted coastal lagoon (Bizerte Lagoon) in comparison to a reference site (the Mediterranean Sea). To our knowledge this is the first study of enzymatic, histopathologic and genotoxic biomarkers using biomarkers from fish liver for assessment in Tunisia coastal waters.
Antioxidant enzyme activities were lower in fish from the polluted site compared with fish from the reference site, suggesting deficiency of the antioxidant system to compensate for oxidative stress. DNA damage was higher in fish from the contaminated site indicating genotoxic effects. The liver histopathological analysis revealed alterations in fish from Bizerte Lagoon. Hepatocytes featured lipid-type vacuolation and membrane disruption. Results suggest that the selected biomarkers in the studied specie are useful for the assessment of pollution impacts in coastal environments influenced by multiple pollution sources.

TU 414
Effects of hypoxia on spermatogenesis and steroidogenesis of marine medaka (Oryzias melastigma) testis: an in vitro study
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Aquatic hypoxia (dissolved oxygen levels less than 2.8 mg O2/L) has become a major concern and more than 200 “Dead Zones”have been identified by the United Nations Environment Program worldwide. Hypoxia has been shown to be an endocrine disruptor, and impairs developmental and reproductive functions in fish. However, the detailed mechanisms of reproductive impairment of hypoxia remain unclear. Using primary cell culture of marine medaka (Oryzias melastigma) testis as an in vitro model, experiments were conducted to decipher the effects of acute hypoxia (2%) on spermatogenesis and steriodogenesis. Hypoxia significantly increased cell death at 24h as measured by Trypan Blue Exclusion ($p<0.001$, n=6) and flow cytometric analysis using propidium iodide (PI) ($p<0.05$, n=5). Flow cytometry showed that hypoxia significantly reduced testicular cell differentiation (spermatocytes and spermatogonia) at 48h ($p<0.05$, n=4), and this was associated with significant down-regulation of expression of steroidogenic genes including CYP11A ($p<0.0005$), 3B-HSD ($p<0.05$) and stAR ($p<0.001$). However, no significant changes in apoptotic cell death were observed following 2% hypoxic treatment at 24 and 48h as analyzed by flow cytometry after labeling with Annexin V and PI. Furthermore, there were no changes in expression of the pro-apoptotic gene BAX and the anti-apoptotic gene BCL2 at these time points. Our results suggest that spermatogenesis and steroidogenesis in fish can be impaired under acute hypoxic conditions, and alterations in germ-line development and steroid hormone regulation are a likely cause for some of the male reproductive abnormalities observed following hypoxic exposure. These molecular changes may potentially reduce reproductive success of males and lead to a decline in natural fish populations.

TU 414
Toxic effects of ZnO nanoparticles towards marine algae Dunaliella tertiolecta
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Dose response curve and population growth rate alterations deriving from the exposure to ZnO nanoparticles of the marine alga D. tertiolecta were evaluated. Bulk ZnO and free zinc ions were also investigated for comparison. Aggregation state and particle size distribution were monitored during the experimental testing time. The results show that nZnO is more toxic (EC50: 2.42 (0.97-5.36) mg L-1, NOEC: 0.01 mg L-1) than its bulk counterpart (EC50: 4.45 (3.45-5.98) mg L-1, NOEC: 1 mg L-1). Cross-referencing the toxicity parameters calculated for ionic zinc (EC50: 0.65 (0.36-0.70) mg L-1, NOEC: 0.01 mg L-1) and the dissolution properties of the ZnO it can be gather that the higher toxicity of nZnO cannot be ascribed exclusively to free zinc ions. Nonetheless growth rates of D. tertiolecta were not significantly affected by nZnO exposure. Our findings suggest that the pristine size of the dispersed particles affect the bioavailability and the overall toxicity

TU 415
PBDEs accumulation in muscle of Whitemouth Croaker (Micropogonias furnieri) exposed to BDE 99 and BDE 153
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Polibrominated Biphenyl Ethers (PBDEs) are flame retardants compounds widely used in plastics, textiles, furniture, electronics, automobiles, and many other materials. Although PBDE are persistent and resistant to degradation, earlier studies have established that some fish can debrominate certain PBDEs congeners. Whitemouth croaker (Micropogonias furnieri) is an important species in the Brazilian fishery industry. Therefore, it is important to understand the accumulation pattern of PBDEs in its muscle. During 30 days, 60 Whitemouth croakers were exposed to BDE 99 and BDE 153 through diet (100 ng fish-1 day-1), followed by a 30 days period of depuration. At days 0, 1, 5, 15, 30, 45 and 60 muscle sample were collects from at least 3 fishes. Overall, the Whitemouth croaker exposure to BDE 99 ended up accumulating BDE 47 in their muscle, indicating their capacity for processing BDE 99. On the other hand, BDE 153 accumulated without any transformation.

TU 416
Inhibition of GST, GPx and AChE activities in marine mussels by exposure to the dissolved polybrominated diphenyl ethers BDE-47
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The environmental presence of polybrominated diphenyl ethers (PBDEs), among which BDE-47 is the most abundant, make toxicity data necessary to assess the hazard risk posed by PBDEs to aquatic organisms. In this study, mussels, Mytilus galloprovincialis, were continuously exposed to 8 µg/L BDE-47 for 30 days. After the exposure phase, the mussels were allowed to depurate in clean sea water for 10 days. Samples were collected at time 2, 5, 9, 15, 20 and 30 days of exposure to toxic. In a second experiment mussels were exposed for 30 days to three BDE-47 concentrations. In both experiments samples were used to measure glutathione S-transferase (GST), glutathione peroxidase (GPx), and acetylcholinesterase (AChE) activities. GST and GPx were inhibited after 20 and 30 days exposure, while AChE was inhibited already from the second day of exposure. After the 10 days recovery period in clean water the three enzymes recovered the initial levels of activity.

TU 417
Differential gene transcription in Poecilia vivipara exposed to copper
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The increasing industrial activities and the use of CuSO4 as a fungicide in agricultural practices, as well as in the control of algae and pathogens in fish culture ponds have raised the copper concentration in aquatic systems. Furthermore, occasional accidents have aggravated this situation introducing substantial amounts of copper into aquatic environments. Copper is accumulated mainly in the liver and gills and excreted via bile and kidneys. The aim of this study was to identify genes differentially expressed in liver of Poecilia vivipara (Guppy) exposed to waterborne copper (20 µg Cu/L) for 24 h, employing the subtractive suppressive hybridization (SSH) method. Results showed that 65 gene fragments were differentially expressed, 37 were up-regulated and 28 were down-regulated. The up-regulated genes were bile salt export, Complement Component 3c, CYP450, enolase, Apolipoprotein B, C1 and E, microsomal glutathione-S-transferase, NAD dehydrogenase, ubiquitin a-52, plasminogen and novel protein. The down-regulated genes were Apolipoprotein A1, Coagulation factor 2, HSP70, Liver-type fatty acid Binding Protein, serine--pyruvate mitochondrial-like. The identified genes are associated with different metabolic functions like biotransformation, protein, lipids and energetic metabolism, indicating the susceptibility and/or molecular responses of this organism to the toxic effects elicited following the trace metal exposure.

TU 418
Spatial distribution and accumulation patterns of cyclic methyl siloxanes (cVMS) in fish from Northern Norway
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Cyclic volatile methyl siloxanes (cVMS) have come under environmental scrutiny in recent years in regards to their potential persistence and bioaccumulative nature. As polymeric ingredients in the synthesis of silicone products, cVMS are categorized as high production chemicals where they are used heavily within the personal care products and cosmetics, as well as other facets of industry (surface treatment agents, plasticizers, construction materials, mechanical fluids). Although the majority of cVMS emissions are to the atmosphere (90%), the remaining emissions are discharged via wastewater effluent into local watersheds and can accumulate in aquatic dwelling organisms. However, little data exists on the spatial distribution of cVMS in the aquatic environment. This study will investigate the spatial distribution of cVMS within Northern Norway and if physiological factors (e.g., age, body length, weight) have an effect on cVMS accumulation within fish. Atlantic cod was collected in 2010 and 2011 near the city of Tromsø (site A) and 30 km north of the city (site B) in Northern Norway. All cVMS were detected in cod livers sampled with highest median concentrations for D5, followed by D6 and D4. Significantly higher median concentrations for octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) were observed in fish collected at site A (D4: 60 ng/g lw; D5: 1380 ng/g lw) compared to site B (D4: 10 ng/g lw; D5: 139 ng/g lw). However, no significant differences were observed in the spatial distribution for dodecamethylcyclohexasiloxane (D6) between sampling sites. Concentration of D5 within cod liver was found to be negatively correlated with age. No correlation was observed between D4 and D6 concentrations and age; however, negative correlations were observed for body length and weight. Concentrations of D4 and D5 significantly decreased away from point sources indicating rapid elimination of these chemicals within fish. No significant differences in concentration for D6 between sampling sites may indicate greater persistence of this chemical to elimination processes. Decreasing cVMs concentrations with age (D5), body length and weight (D4 and D6) may be an effect of enhanced metabolic activity and/or growth dilution within older fish.

TU 420
Organochlorine pollutants in oceanic migratory birds: influence of body condition in PCBs and DDTs concentrations among different tissues
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Albatrosses and petrels (Procellariiformes) are migratory oceanic birds of considerable conservational interest. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were assessed in the subcutaneous fat, liver and muscle of one hundred birds belonging to eight species of Procellariiformes collected during their migration period in southern Brazil, one of the most important feeding areas for these species. The influence of body condition during the migration period in the distribution of organochlorine contaminants was evaluated, showing that it is a significant factor in the variation and redistribution of these compounds in the tissues of these birds. Birds with good body condition presented PCBs and DDTs concentrations in the fat tissue significantly higher than those found in the liver and muscle tissue. On the other hand, 60% to 70% of the specimens with signs of starvation such as the depletion of fat reserves had higher DDTs and PCBs concentrations in the liver than in the fat tissue, although the difference in the concentrations of OCs between these two tissues did not achieve statistical significance. These findings suggest a tendency toward an increase in the concentration of these compounds in the liver and muscle of birds with poor body condition. The concentration of these compounds is influenced by the body condition of the bird at the time of collection and the analysis of different tissues may generate different findings regarding contamination in birds. Lipid reserves are extremely important to the achievement of high energy cost activities to seabirds, mainly those with extended reproductive periods and that spend most of their lives at sea migrating long distances, such as Procellariiformes. The process of maintenance and utilization of lipid reserves during the life cycle of the bird imply in a frequent redistribution of OCs in the organism, when the negative effects of these contaminants may be manifested.

TU 420
Development of appropriate bioassay and statistical methods for determining survival sensitivities of Antarctic marine biota to metal exposure
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Developing water quality guidelines for Antarctic marine environments requires understanding sensitivity of biota to contaminant exposure. Toxicity tests with Antarctic invertebrates have shown response times to metal exposure takes far longer than the durations of standard bioassay methods. Test methods which address the biological characteristics of these cold climate species and theenvironment need to be developed. This study investigated the effects of five metals on behaviour and survival of an Antarctic amphipod, Orchomenella pinguides, over 30 day exposures. Multiple observations were made assessing mortality rate response of this species to metal exposure. As traditional bioassay methods use probit analysis for individual period endpoints treated independently, a new statistical model that combines bioassay model with a survival time model was developed. Interval-censored survival times were modelled using a generalised additive model (GAM) with multiplicative effects for concentration level used to incorporate the bioassay component. The time period by concentration level interaction was included as a random effect term with this mixed model version of the GAM (called a GAMM) allowing model adequacy to be investigated. The advantages of this approach are that (i) it smooths through noisy periodic mortality data "gaining strength"by estimation of the trend across multiple observation periods exploiting all data simultaneously, (ii) the simple multiplicative model, if adequate, does not allow cross-overs of time trends across concentrations that make interpretation difficult, and (iii) it allows the uncertainty in estimates of lethal concentrations to be more adequately modelled.
Response times vary between metals with amphipods exposed to copper producing behavioural changes and mortality at a faster rate than exposure to cadmium, lead or zinc. Nickel did not affect the behaviour or survival of the amphipod during the 30 day exposures despite using concentrations > 1mg/L. Results from comparisons of both approaches indicate that standard bioassay methods have serious limitations when applied to Antarctic species which are generally long lived, have longer developmental times, and slower metabolic processes and potentially metal uptake than species from other climatic regions. Recommendations are made for further toxicological assessments for the future development of Water Quality Guidelines to protect Antarctic marine environments.

TU 421
PAH in fish: biological tool for Guanabarab Bay environmental evaluation
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